NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT (U.S.)

ANNUAL REPORT OF INTRAMURAL RESEARCH

October 1, 1988 through September 30, 1989

TABLE OF CONTENTS

CELL BIOLOGY AND METABOLISM BRANCH (CBMB)

Z01 HD 01600-05	Biochemical Basis of T Cell Activation Larry E. Samelson, M.D.
Z01 HD 01601-05	Molecular Aspects of the Regulation of the Human Transferrin Receptor Joe B. Harford, Ph.D.
Z01 HD 01602-05	Regulation of Intracellular Iron Metabolism Richard D. Klausner, M.D.
Z01 HD 01604-04	Interleukin-2 Receptor - Structure, Function, and Regulation Warren J. Leonard, M.D.
Z01 HD 01605-03	T-Cell Antigen Receptor - Structure, Biosynthesis and Cell Biology Richard D. Klausner, M.D.
ZO1 HD 01606-01	The Biology of Early Organelles of the Secretory Pathway Richard D. Klausner, M.D.

NICHD Annual Report
October 1, 1988 to September 30, 1989

Cell Biology and Metabolism Branch

INTRODUCTION

The Cell Biology and Metabolism Branch has continued its work in the areas of iron metabolism, gene regulation, immunology and cell biology. The work of the laboratory has been divided into six projects which are listed below. This past year has been an extremely productive one for the Cell Biology and Metabolism Branch. This productivity is attested to by the more than 55 publications produced over the past twelve months. The laboratory has maintained its clinical activity in studying the problems of diseases of human metal metabolism. In addition, the laboratory has continued its interest in the cell and molecular biology of the human immunodeficiency virus (HIV), the etiologic agent responsible for AIDS. During the past year there has been a major expansion in the physical facilities of the branch to include an additional, neighboring building containing 3,000 square feet of newly renovated laboratory space. The six projects that encompass the branch's work are as follows:

Group Leader	Group
Richard D. Klausner	Regulation of Intracellular Iron Metabolism
Joe B. Harford	Molecular Aspects of the Regulation of the Human Transferrin Receptor
Lawrence E. Samelson	Biochemical Basis of T Cell Activation
Richard D. Klausner	The T Cell Antigen Receptor - Structure, Biosynthesis and Cell Biology
Warren J. Leonard	Interleukin-2 Receptors - Structure, Function and Regulation
Richard D. Klausner	The Biology of Early Organelles of the Secretory Pathway

Molecular basis of human iron metabolism

We have continued the long term interest of this laboratory in understanding the basis of iron metabolism. The importance of iron metabolism in human biology becomes apparent in many areas of research. Because iron is absolutely essential and perhaps rate limiting in the growth of cells, the ability of cells to regulate and assure iron uptake is strictly correlated with the proliferative rate of cells. This is true during development, in normal differentiation, proliferation, inflammation, the immune response, wound healing and neoplasia. In addition to its correlation with cellular growth, iron is absolutely required for maintenance of baseline metabolic activities. Thus, obtaining sufficient iron is critical for cell health of both the individual cell and the entire organism. This importance is reflected in the wide range of clinical consequences of iron deficiency, a common clinical disorder. In contrast to iron deficiency, where not enough iron leads to pathologic consequences, too much iron is extremely toxic and will lead to cell death. The inability to regulate the uptake of iron underlies one of the most common genetic diseases of man, hereditary hemochromatosis. This disease, which affects one in 400 people in our population, is due to a failure to regulate iron uptake and results in a tremendous amount of morbidity and, if untreated, mortality. The Cell Biology and Metabolism Branch is at the forefront of studies aimed at elucidating the molecular basis of iron metabolism. Our studies continue to focus on how genes are regulated by iron. We are emphasizing this aspect of iron metabolism because it is the ability of iron metabolism to be regulated in a homeostatic way that underlies cellular mechanisms that attempt to prevent both iron deficiency and iron toxicity. The work on the regulation of proteins involved in human iron

metabolism has not only elucidated that specific problem but has uncovered a major new area of post-transcriptional gene regulation. The identification of specific cis-acting RNA elements and regulatory trans-acting RNA binding proteins has placed post-transcriptional gene regulation on a firm and detailed molecular basis. We now know that there are two critical elements that underlie the iron regulatory system: the transferrin receptor, along with its ligand, transferrin, and ferritin. Work from this laboratory has brought us very close to a molecular understanding of how these genes are regulated in response to iron. Ferritin is regulated at the translational level such that when there is little iron there is very little translation, and when there is a large amount of iron there is a larger amount of translation. The transferrin receptor is predominantly regulated at the level of the half-life of the mRNA encoding the protein. In contrast to ferritin, when there is little iron, mRNA levels for the transferrin receptor increase due to a longer half-life and when there is more iron the half life of the mRNA shortens, resulting in a drop in receptor number and less iron uptake into the cell.

Work in this laboratory over the past several years has allowed us to define two molecular elements underlying the regulation of these two genes. One is an RNA element, a sequence found within the messenger RNA molecules encoding both ferritin and the transferrin receptor. This element, which we refer to as an iron-responsive element or IRE, is a small stem-loop structure which endows iron sensitivity to the regulation of the fate of the RNA. A single IRE present in the 5' untranslated region (5' UTR) of ferritin mRNA molecules is entirely responsible for the ability of iron to regulate the translation of the ferritin message. The region of the mRNA of the transferrin receptor responsible for its regulation in response to iron are contained within the 3' untranslated region. This region has been shown to contain 5 RNA motifs with sequences resembling the ferritin IRE.

The second molecular element involved in the regulation and expression of these iron related genes is a cytosolic protein (proteins) which specifically binds with high to the IRE RNA regulatory element. This protein has been identified by the use of a combination band shift and RNAse protection assay. As predicted from sequence analysis, the same cytosolic binding protein interacts with the IRE's found in the ferritin 5' UTR and with IRE's identified in the regulatory region of the 3' UTR of the transferrin receptor. These observations have allowed us to formulate a unifying model to explain the apparent disparate regulation of these two genes. According to this model, when there is little iron around, the IRE binding protein becomes activated and binds to the IRE. If the IRE is in the 5' UTR of the gene, the binding of that protein will sterically block the initiation of translation. For the transferrin receptor mRNA the situation is a bit more complex. In order to explain the regulation of message half-life by iron via the IRE and its interaction with the binding protein, the following can be proposed: Once again, the absence of iron activates the IRE binding protein to bind to the RNA regulatory element. We have provided data for the existence of two functional elements in the regulatory region of the TfR mRNA. The first endows the region with with iron responsiveness and can be entirely ascribed to the ability to bind to the IRE binding protein. The second is a functional element that has no bearing on the interaction with the iron dependent trans factor, but is an mRNA instability determinant. It is clear that these two elements are intimately intertwined structurally and it is the dissection of these two elements that allows us to propose a model for this regulated stability. In the absence of iron the IREs in the regulatory region are bound by the IRE-BP and this blocks the function of the intrinsic RNA instability element. Thus, in the absence of iron, the mRNA is stabilized. In this way the increased binding of the IRE binding protein in the absence of iron can explain both the decreased rate of biosynthesis of ferritin and the increased rate of biosynthesis of the tranferrin receptor. Support for this model has been provided by the finding that when cells are treated with an iron chelator in order to starve them of accessible iron, the specific activity of the IRE binding protein in the cytosol is considerably increased. In addition to this model for how the IRE binding protein could regulate iron-responsive genes at the post-transcription level, we are beginning to unravel the actual biochemical mechanisms by which the IRE binding protein may both respond in its activity to iron levels and interact with the IRE.

Major advances in our understanding of the IRE-BP has accrued over the past year. First of all, we have purified the IRE-BP to homogeneity using a novel RNA affinity purification scheme. The protein has been purified in significant amounts, and has been subjected to extensive peptide amino acid sequencing. Using the extensive protein sequence that we now have available, we are attempting

to clone the cDNA encoding this protein. The protein is a relatively abundant 90 kD protein. In addition, we have established what we believe to be a novel biochemical regulatory mechanism to explain the iron sensitivity of the interaction of the IRE-BP with its cognate RNA elements. We have found that the IRE-BP exists in two binding states, a low affinity state (kD = 5 nm) and a high affinity state (kD = 10-30 pm). In the absence of iron, a large fraction of the total population is present in the high affinity state. Conversely in the presence of iron, the vast majority (greater than 99%) is found only in the low affinity binding state. The switch between the two affinity states appears to be a reversible redox reaction within the protein. Thus, at least one critical pair of sulfhydryls, when oxidized, results in a protein taking on only the low affinity state. When this disulfide is reduced to produce the free sulfhydryls, the protein is in the high affinity binding state. These two redox states of the protein are determined within the cell in response to changes in iron status and then are each quite stable. This is the first example of a cytosolic protein being regulated by this type of "sulfhydryl switch."

In addition to the tranferrin receptor, transferrin and ferritin, other critical genes are undoubtedly involved in human cellular and total body iron metabolism. The identification of these genes, however, is a major problem. Because the essentials of iron metabolism is likely to be similar in all organisms, be they primitive prokaryotes, yeast, or higher eukaryotic and mammalian cells and organisms, we have embarked on a new approach to solving this problem. Accordingly, we are examining iron metabolism in the yeast Saccharomyces cerevisea. We have shown that this organism is absolutely dependent on the uptake of environmental iron for health, growth and proliferation. We are using both biochemical, protein chemical and, most importantly, genetic approaches in order to identify genes and gene products of this yeast that are involved in iron uptake. In particular we have identified an iron reductase gene present in the membrane of yeast cells that is responsible for the initial events in the transport of iron across cellular membranes. This ferric reductase gene has now been isolated and cloned and shown to be highly regulated in response to a variety of environmental changes including nutrient deprivation, oxygen status and, importantly, iron status. Transmembrane uptake of iron is one of the major gaps in our understanding of human iron metabolism. We believe that obtaining the yeast gene encoding this iron reductase will allow us to identify the corresponding higher eukaryotic gene.

One of the goals of all of these studies is the application of the insights gained to diseases of iron metabolism. We continue to have a very active clinic in which we examine patients with hereditary hemochromatosis and establish continuous cell lines from their peripheral blood lymphocytes. This provides us with not only cells from these patients with which to study physiologic abberations, but also a source of genetic material to look at the molecular basis of possible defects underlying hereditary hemochromatosis. In addition, these studies are giving us unique insights into specific mechanisms of gene regulation in human cells. They are also providing us with useful applications of recombinant DNA technology. Accordingly, the discovery of the IRE has led to our patenting this new type of genetic element for possible use as part of an iron-regulated expression vector system.

RECEPTORS OF THE IMMUNE SYSTEM

The T Cell Antigen Receptor

Largely due to the work of this laboratory, the T cell antigen receptor is now understood as one of the most complex integral membrane receptor molecules. This complexity applies both to subunit structure and biochemical function. We now know that the receptor is composed of at least seven different proteins that are assembled in complexes consisting of seven, nine or more chains. We have named the two most recently defined chains of this receptor complex, the zeta and eta chains. Both the murine and the human zeta chain genes have been cloned by us in the past year and this has led to a complete elucidation of the structure of the protein. The zeta chain comes in two different forms, as a homodimer and as a heterodimer. In the latter situation it is linked to a somewhat larger chain which we have called eta. The frequency of finding eta is only about one-tenth the frequency of finding zeta and thus the hetrodimer is most likely only found in the minority of surface receptors. One of the goals of our studies is to correlate the structure of the receptor with its function. We define receptor function by examining both the very proximal biochemical events that

ensue upon receptor stimulation or the resulting phenotypic changes in cells (generally referred to as cell activation). In order to make these structure/function correlations we are taking two approaches: 1) the isolation of mutants or variants of antigen specific T cell hybridomas; and 2) the reintroduction of genetically altered T cell receptor subunits into deficient T cell lines. Over the past year much progress has been made using both of these approaches. In particular functional consequences of failing to synthesize either the zeta or the eta chain have been examined. The absence of the eta chain has no effect on the surface expression of the receptor complex. However, it does seem to be correlated with severe functional deficiency. We have previously shown that the T cell antigen receptor functionally couples to at least two cellular biochemical pathways. In one, phosphorylated phosphatidylinositides are broken down in response to receptor activation, leading to the release of water soluble inositol phosphates and diacylglycerol. The result of this is the activation of protein kinase C and the mobilization of intracellular calcium. The other pathway involves the activation of a non-receptor tyrosine kinase or tyrosine kinases which result in the tyrosine phosphorylation of a number of cellular substrates (see below). In the absence of eta, receptor mediated tyrosine kinase activation is maintained while the ability to couple the phosphatidylinositide (PI) pathway is lost. We have established a variety of eta-negative cell lines. These lines have allowed us to solidify our model for two functional classes of T cell receptors, one containing zeta-zeta, and one containing zeta-eta. As we have previously seen, zeta-eta containing receptors are responsible for coupling to PI metabolism. Interestingly, cells that have no eta are capable of making normal amounts of IL-2. In contrast, cells that have no eta are not capable of undergoing receptor-mediated cell death. This is particularly intriguing because we believe that cell mediated apoptosis is currently the best model for negative selection in the thymus. The finding that one did not need PI turnover for IL-2 production raised the question of whether the tyrosine kinase pathway is capable of stimulating IL-2 production. One approach to testing this was the isolation of stable transfectants of 2B4 T cell hybridomas containing and expressing the v-src oncogene, an active tyrosine kinase. We noted that when this took place, a variety of intracellular substrates normally phosphorylated in response to T cell receptor engagement were now constituently phosphorylated. More importantly, these cells constituitively produced IL-2. When the rate of transcription of the transfected gene was increased, the rate of production of IL-2 was likewise increased. This is the first demonstration of the ability to mimic a particular cellular activation pathway by the introduction of an exogenous unregulated tyrosine kinase.

Structure/function correlations for the T cell antigen receptor in the lab involve the characterization of the eta chain and mutagenesis of the zeta chain followed by reconstitution of zeta deficient T cell clones. We have extensively characterized the biochemical nature of the eta chain and have finally developed antibodies that recognize both the human and murine eta chain. In addition, we have begun mutating the zeta chain by site-directed mutagenesis, followed by reintroduction of the mutated zeta chain to reconstitute zeta deficient T cells. This has resulted in the demonstration that truncation of the cytoplasmic tail of zeta essentially destroys the ability of information to flow from the alpha-beta ligand recognition chains to the coupling CD3 chains. These studies represent the first attempts to molecularly dissect information flow within this complex signalling structure.

As stated above, multiple kinases are activated in response to the stimulation of the T cell antigen receptor. We believe that understanding the nature of these kinases, their pattern of activation, their pattern of regulation, their interaction, and their relevant cellular substrates would be absolutely critical to our ability to understand and possibly manipulate the immune response. We have defined a set of cellular substrates that are tyrosine phosphorylated in response to receptor activation. Recent data suggests that there may be two different sets of substrates phosphorylated in response to receptor occupancy and that these two sets may be the targets of two different tyrosine kinases. One set includes the rapid phosphorylation (within seconds) of a cytosolic protein called pp62. After pp62 is phosphorylated the zeta chain of the T cell receptor is phosphorylated. Another set of substrates has been defined which appear to be extremely susceptible to dephosphorylation by a tyrosine phosphatase within the cell. In order to see the phosphorylation of this set of substrates, one needs to examine the cell in the presence of the tyrosine phosphatase inhibitor. The pharmacologic characteristics of the phosphorylation of these two sets of substrates are quite distinct. We are currently attempting to purify the pp62 tyrosine kinase substrate as it may be the tyrosine kinase linked to the T cell receptor. We have also examined changes in the major T cell membrane tyrosine phosphatase, CD45, in response to receptor activation. We have defined a novel and intriguing

cellular activation pathway that changes the intracellular distribution of CD45 in response to receptor activation. We are currently pursuing this phenomenon. What is intriguing about this phenomenon is that it represents the first example of a change in the residency time of a protein in an intracellular organelle in response to external signalling. The role of this redistribution in T cell activation is currently being examined. In response to receptor stimulation an additional cytosolic kinase is phosphorylated. This is the cellular homologue of the raf-oncogene. This kinase is a serine/threonine specific kinase and every molecule of c-raf within the T cell is phosphorylated, perhaps on multiple sites, in response to receptor activation. We are currently exploring the regulatory consequences of the phosphorylation of this proto-oncogene.

The Human Interleukin-2 Receptor and T Cell Activation Genes

T cell activation is accompanied by a genetic program whereby a number of genes are turned on in a predictable and defined pattern. This pattern of genetic program expression is stereotyped both in terms of kinetics, order of genes, and the groups of genes involved. One of the most important sets of genes that are turned on involve the interleukin-2 system. Interleukin-2, or T cell growth factor, provides autocrine, paracrine and endocrine stimulation for the proliferation of T cells. In order for these T cells to respond to this growth factor they must express a receptor that is specific for it. The IL-2 receptor group has continued to examine two aspects of its biology. The first of these is directed towards determining the genetic elements that are involved in the carefully regulated expression of the interleukin-2 receptor alpha subunit gene. Using a variety of techniques for the study of 5' genomic flanking elements as transcriptional elements as well as techniques aimed at looking at specific DNA protein interactions, this group has come a long way in elucidating the sequences involved in the expression of this gene. In addition to the regulated expression of this gene during the activation of T cells, the gene for the IL-2 receptor alpha chain is also highly expressed in an apparently unregulated fashion in cells infected with and transformed by the human T cell leukemia virus, HTLV-I. Again, this group has been defining those elements that appear to be responsive to the expression of gene products encoded by this virus. One area of some interest that has been focused on over the past year is the potential role of a particular DNA binding protein, first described as an enhancer element for the kappa immunoglobulin light chain gene. A consensus sequence that defines this element binds a particular transcription factor called NF-kappa-B. It appears that the same motif is present and capable of binding an NF-kappa-B molecule (or molecule closely related to it) in the regulatory region of the IL-2 receptor gene. The role of this transcriptional factor in the expression of the IL-2 receptor alpha chain gene is being explored. This is of particular interest because of the role of these sequences and this factor in the activation of the human immunodeficiency virus, HIV. In addition to the NF-kappa-B binding site, a variety of other protein binding sites have been identified within this small region critical to the expression of the IL2R-alpha gene. These include the previously described serum response factor, SP1 and one or two newly defined binding proteins.

Another aspect of the biology of the interleukin-2 receptor explored by this group is the role of the beta subunit of the receptor (p70) first described by this group two years ago. The ability of the beta subunit to exist on the cell surface in the absence of the alpha subunit and to serve as a receptor in those cells has been demonstrated. This is true of a variety of peripheral blood mononuclear cells such as large granular lymphocytes and the precursors of LAK cells and NK cells. That the beta subunit can be a receptor, or part of a receptor, in the absence of the alpha subunit is demonstrated by both its ability to bind IL-2 and, in fact, mediate biologic responses to that binding. The beta chain of the IL-2 receptor is not restricted to T cells but can be induced to exist on the surface of both peripheral blood B cells and monocytes. The potential roles in immunobiology of this receptor subunit is only beginning to be explored. The recent cDNA cloning of the beta chain of the IL-2 receptor has allowed this group to isolate full-length cDNAs, as well as genomic clones, which are beginning ot be analyzed for critical regulatory elements.

Finally, this group has been engaged in characterizing other genes that are rapidly turned on in response to T cell activation. One of these, which has been referred to as Act-II, is rapidly turned on in both T cells and other mononuclear cells in response to stimulation. The gene and cDNA encoding this protein have been characterized. The cDNA predicts a small secreted protein, suggesting that this may be a new lymphokine. In addition, this protein has recently been expressed

in a variety of systems including a bacculovirus expression system in cultured insect cells. This system has allowed the secretion of a large amount of protein to enable more biochemical analysis as well as functional studies of this novel T cell activation gene product.

Cell and Organelle Biology

Work in this area of biology in the laboratory has exploded over the past year, necessitating the formation of a new project. Much of this work, stemmed out of initial studies on the problem of the assembly and intracellular transport of the seven membered T cell receptor complex. These studies led to the formulation of the concept of "architectural editing", an overall set of cellular processes that relate the fate of newly synthesized multicomponent complexes within the cell to the structure of those complexes. We have been examining the signals used in architectural editing, the intracellular pathways by which incorrect complexes are edited out of the cell and the recent finding that architectural editing is not simply a mechanism of quality control for abnormal proteins but for the regulated expression of a variety of protein complexes by the cell. Because so much of architectural editing takes place within the endoplasmic reticulum, we have been focusing our attention on new structures and functions within that complex organelle.

ER Degradation. In last year's Annual Report we reported our description of a new degradative pathway within the cell responsible for the degradation of newly synthesized proteins that are deemed to be structurally abnormal by the cell. This year we have focused on trying to understand one of the most striking aspects of ER degradation - its specificity. We identified this initially by studying the fate of subunits of the seven-membered T cell antigen receptor when retained within the endoplasmic reticulum. We found that three of the subunits were selectively and rapidly degraded by this non-lysosomal degradative system while the remaining subunits were stable for long periods of time within the organelle. The degraded subunits include the alpha chain, the beta chain and the delta chain while the gamma, epsilon and zeta chains are stable. One observation that may be pertinent to the selectivity is that the complex, if prevented from leaving the ER, begins to disassemble. This disassembly can be blocked by relatively minor reductions in the temperature. Interestingly, when the temperature is reduced to prevent disassembly, then no degradation is seen. The degradation itself appears to be temperature sensitive and all ER degradation is blocked at temperatures below about 15°C. Thus, if the alpha chain or alpha-beta heterodimer is expressed in fibroblasts in the absence of any other subunits, ER degradation takes place at all temperatures above 16°C at which point there is an apparent phase transition in the activation energy for degradation. If, however, we look at the ER degradation of alpha-beta in T cells in which full assembly occurs in the ER preceeding degradation, then the temperature coefficient for degradation changes dramatically, there is no longer a phase transition and the degradation is dramatically inhibited by simply dropping the temperature to 30°C. The relationship between assembly and degradation within the ER was further examined when we asked why the CD3-gamma chain was not degraded. This was puzzling because CD3-gamma is so similar to the CD3-delta which is degraded. To test this we transfected the cDNA encoding CD3-gamma into fibroblasts. We noticed that in this case gamma did not leave the ER and was rapidly degraded by ER degradation. In contrast, if we cotransfected the epsilon chain, epsilon-gamma complexes were formed and this prevented the degradation of gamma. When epsilon is transfected alone, it is not subject to ER degradation.

A more definitive approach to the selectivity of degradation has been to dissect the alpha chain of the T cell receptor and to ask the question whether any region of this protein contains the information for ER degradation. When the transmembrane and five amino acid cytoplasmic tail is removed from alpha, leaving a truncated, potentially secreted protein, we observed that this truncated protein, when expressed in cos cells, is not secreted, remains in the ER but is not rapidly degraded. To test whether the relatively short sequence removed in this experiment contained information to target other proteins for ER degradation, the transmembrane and cytoplasmic domains of alpha were fused with the lumenal domain of the IL-2 receptor Tac antigen. The IL-2 receptor, when expressed in cos cells is not degraded even if it is forced to remain in the ER by treatment with the drug, Brefeldin A. Furthermore, the truncated form is rapidly secreted and, again, if it is retained in the ER by treatment with this drug, there is no ER degradation. However, when the chimeric protein is made, it is both retained in the ER and targeted for extremely rapid ER degradation.

Membrane Dynamics in the Secretory System. In looking for pharmacologic reagents that might block functions associated with the endoplasmic reticulum, we noted studies performed and reported by a group of Japanese researchers on the fungal antimicrobial agent, Brefeldin A. This is a prostaglandin-like heterocyclic lactone made by a variety of fungi.. It had been reported to block secretion out of the ER. We began examining its effect on the cell and noted a remarkable finding. When cells were treated with this drug, within minutes the Golgi began disassembling and by ten minutes, integral membrane proteins of the Golgi were now found uniformly through the endoplasmic reticulum. This was the first observation of the ability to specifically return Golgi proteins to the ER. The ER now functioned as a mixed ER and Golgi and carbohydrate side chains of glycoproteins retained in this organelle were now processed as if they had reached the Golgi. The possible presence of a retrograde pathway within the secretory system challenges many of our notions of the structure and function and dynamics of the secretory pathway. This retrograde movement induced by Brefeldin A has been more carefully studied. It is a temperature sensitive process that seems to be divided into two discrete steps. At temperatures below 16°C Brefeldin A has no effect on the Golgi. At temperatures between approximately 18-22°C the Golgi appears to fuse into a large vacuolar structure that gives off long strings of necklace-like tubulovesicular extensions. However, at this intermediate temperature, no apparent fusion with the ER is observed. At temperatures above 22°C the Golgi distributes through the ER. A striking observation was that agents that disrupt microtubule structure or function, such as microtubule depolymerizing agents (nocodazole) or others (griseofulvin), block the movement of the Golgi back to the ER. When cells are pretreated with nocodazole and then given Brefeldin A, the Golgi seems to move into large scattered structures that interestingly colocalize with antibodies directed against transition vesicles, organelles that are believed to sit in the secretory pathway between the ER and the Golgi. In contrast to this retrograde movement, anteriograde movement out of the ER and back into the Golgi is unaffected by these agents that disrupt microtubule function. We next tested whether nocodazole would block a recycling pathway that operates either between the cis-Golgi or pre-Golgi compartment and the endoplasmic reticulum. The remarkable findings have been that proteins that are retained in the ER, either due to treatment with Brefeldin A or because of the presence of the KDEL "ER retention" signal, rapidly accumulate in this intermediate compartment upon treatment with nocodazole. This is the first evidence for the structure and identify of an ER recycling/retrieval pathway.

The Role of the ER in Antigen Presentation. We have used the ability to completely block movement out of the endoplasmic reticulum with the drug, Brefeldin A, to test whether the ER is involved in the presentation of antigen. Our first studies examined the presentation of an endogenous antigen, an internal peptide of the influenze virus matrix protein, by class I major histocompatability complex (MHC) molecules. This was done using a human B cell line as a target for cloned human T cell killer lines. We found that Brefeldin A, while not blocking viral protein synthesis, completely blocked the ability to present endogenous antigens by the class I pathway. It did not block the ability of these same cells to present the processed peptide when added to the outside of the cell. In addition, it did not affect the ability of class II MHC molecules to present their peptides and nor did it block the ability of antigen presenting cells to process exogenous antigens to be presented by class II molecules. However, we have recently found that the ability to present endogenous antigens by class II molecules, is identically blocked by Brefeldin A as is the class I endogenous pathway. This work has allowed us to propose a new model for the cell biology of antigen presentation, as well as to describe the possibility of pharmacologic agents blocking this process.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01600-05 CBMB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biochemical Basis of T Cell Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.E. Samelson	Med. Officer (Res.)	CBMB, NICHD
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	J.J. O'Shea	Expert	CBMB, NICHD
	J.N. Siegel	Senior Staff Fellow	CBMB, NICHD
	P. Garcia-Morales	Visiting Fellow	CBMB, NICHD
	E.T. Luong	Technician (Chem.)	CBMB, NICHD
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Cell Biology and Metabolism Branch

SECTION
Section on Organelle and Receptor Structure and Function

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NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Activation of the multicomponent antigen receptor (TCR) in T cells results in rapid activation of protein kinase C (PKC) and an unidentified tyrosine kinase. The tyrosine kinase pathway has been studied by: 1) infecting T cells with the v-src kinase; 2) characterizing tyrosine kinase substrates; and 3) evaluating the kinases associated with the TCR and the T cell specific CD4 and CD8 molecules. One substrate of protein kinase C in T cells has been identified. The serine/threonine kinase c-raf is phosphorylated by activated PKC resulting in a change in c-raf enzymatic activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01601-05 CBMB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Aspects of the Regulation of the Human Transferrin Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Others:	J.L. Casey	IRTA Fellow	CBMB, NICHD
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COOPERATING UNITS *(if any)*

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LAB/BRANCH
Cell Biology and Metabolism Branch

SECTION
Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	4	0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Post-transcriptional regulation of transferrin receptor mRNA levels by iron is mediated by a portion of the 3' untranslated region (UTR) of the mRNA. We have previously shown that a 678 nucleotide fragment of the 3' UTR contains the regulatory element(s). Within this region are five RNA structures which resemble the iron responsive element (IRE) in the 5' untranslated region of the ferritin mRNA, which is regulated translationally by iron. The IRE's from the ferritin and transferrin receptor mRNA's compete in an in vitro assay for interaction with a cytoplasmic protein; the activity of this IRE-binding protein is dependent upon the iron status of the cells. Thus, despite the differences in the translational regulation of ferritin and the regulation of TfR mRNA levels, these two post-transcriptional regulatory mechanisms share a cis-acting RNA element (IRE) and a trans-acting cytoplasmic protein that interacts with the two mRNA's. We are attempting to understand more fully the mechanism of the regulation of TfR expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01602-05 CBMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Intracellular Iron Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.D. Klausner	Head	CBMB, NICHD
Others:	T.A. Rouault	Sr. Staff Fellow	CBMB, NICHD
	M.W. Hentze	Visiting Associate	CBMB, NICHD
	D. Haile	Med. Staff Fellow	CBMB, NICHD
	C. Tang	IRTA Fellow	CBMB, NICHD
	A. Dancis	Med. Staff Fellow	CBMB, NICHD
	J.G. Barriocanal	Visiting Fellow	CBMB, NICHD
	J.B. Harford	Senior Investigator	CBMB, NICHD

COOPERATING UNITS *(if any)*

American Red Cross, Rockville, MD (W. Burgess)

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.33	5.33	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is aimed towards a molecular understanding of the basis of intracellular iron metabolism. A molecular genetic examination of human ferritin has set the basis for defining a cis/trans regulatory model for the post-transcriptional regulation of this critical protein of intracellular iron metabolism. Iron regulates the translation of the mRNA encoding ferritin by virtue of its ability to alter the binding activity of a cytosolic protein that binds to a specific RNA sequence contained within the ferritin message. A complete description of this RNA regulatory element, the RNA binding protein, and how iron regulates their interaction will provide the first complete description of a translational control system in higher eukaryotic cells. In order to elucidate previously unknown components of the human cellular iron metabolism, we have established the mechanism for the uptake of iron in the genetically manipulatable simple eukaryote, Saccharomyces cerevisiae. This has led to the identification of a reductase/transporter system to explain the transmembrane, regulated uptake of iron in this organism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01604-04 CBMB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Interleukin-2 Receptors - Structure, Function, and Regulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W.J. Leonard	Med. Officer (Res.)			CBMB, NICHD
Others:	S.L. Cross	Adjunct Scientist	D.G. Roman	Med. Staff Fellow	CBMB, NICHD
	J.R. Gnarra	IRTA Fellow	M. Sharon	Sr. Staff Fellow	CBMB, NICHD
	N.F. Halden	Technician (Biol.)	C. Spencer	Adjunct Scientist	CBMB, NICHD
	B.B. Lin	Adjunct Technician	M. Toledano	Visiting Fellow	CBMB, NICHD
	M. Napolitano	Visiting Fellow			CBMB, NICHD

COOPERATING UNITS *(if any)*
Baylor College of Medicine, Houston, TX (N.T. Chang); Division of Virology, FDA (J.P. Siegel); Whitehead Institute for Biomedical Research, Cambridge, MA (M.J. Lenardo); Brigham and Women's Hospital, Boston, MA (J.S. Pober); American Red Cross, Rockville, MD (W. Burgess)

LAB/BRANCH
Cell Biology and Metabolism Branch

SECTION
Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8	7	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The human interleukin-2 (IL-2) receptor (IL2R) is being studied in order to understand specific critical components of the T cell immune response in normal and neoplastic cells. The approaches used are based on (1) biochemical analysis of high, intermediate, and low affinity human and murine IL2Rs; (2) characterization of transcription regulatory sequences in the IL2R-alpha gene and associated DNA binding proteins; (3) isolation of full length IL2R-beta chain cDNAs and characterization of patterns of IL2R-beta mRNA and protein expression; (4) isolation of genomic clones corresponding to the IL2R-beta gene with subsequent characterization of the promoter region. We were the first to identify the existence of a 65 to 77 kD glycoprotein (p70, IL2R-beta) which is a component of the high affinity human IL2R, distinct from IL2R-alpha (p55, Tac antigen), and which can bind IL-2. IL2R-beta mediates the generation of LAK cells and IL-2 induced augmentation of NK activity. Further it is present on at least a subpopulation of resting T cells, and can be induced on B cells and monocytes. We have partially mapped the region of the IL2R-alpha gene necessary for transcriptional activity using IL2R-alpha-CAT constructs in transfection experiments. We now have delineated an enhancer-like positive regulatory region within the IL2R-alpha gene. This region contains binding sites for two previously identified factors, NF-KB and serum response factor (SRF), and binding sites for a factor that may be SP1 and at least one additional previously uncharacterized factor denoted NF-IDR1. The regulation of expression of the IL2R-alpha gene appears to depend on both positive and negative regulatory elements. We have also identified a new gene, Act-2, which is induced in T cells within 15 min of exposure to PHA, reaching maximal levels of mRNA expression in 4 h and declining significantly by 16 h. The Act-2 gene has been cloned and promoter region isolated. This gene encodes a secreted product which has been expressed using a baculovirus expression vector system. Act-2 protein has been purified and radiolabeled and a putative Act-2 receptor identified. Efforts to study its biological function(s) and molecular regulation are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01605-03 CBMB

PERIOD COVERED: October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: The T Cell Antigen Receptor - Structure, Biosynthesis and Cell Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.D. Klausner	Head	CBMB, NICHD
Others:	A.M. Weissman	Sr. Staff Fellow	CBMB, NICHD
	M. Baniyash	Visiting Fellow	CBMB, NICHD
	D.G. Orloff	Med. Staff Fellow	CBMB, NICHD
	V. Hsu	IRTA Fellow	CBMB, NICHD
	S.J. Frank	Med. Staff Fellow	CBMB, NICHD
	N. Manolios	Adjunct Scientist	CBMB, NICHD
	J.S. Bonifacino	Visiting Associate	CBMB, NICHD

COOPERATING UNITS *(if any)*

Biological Response Modifiers Program, NCI (J. Ashwell); Divison of Cancer Biology and Diagnosis, NCI (A. Singer)

LAB/BRANCH: Cell Biology and Metabolism Branch

SECTION: Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goals of this group are to identify the structure and the regulated expression of the T cell antigen receptor complex. In addition, an important component to the work of this group is to utilize mutants combined with recombinant DNA technology to begin to establish structure/function relations for this important immunologic receptor. The structure of two different receptor complexes on the surface of T cells have been defined. Both complexes contain the alpha and beta heterodimeric antigen recognition structures non-covalently coupled to the three membered CD3 complex. The two classes of receptors are distinguished by their containing either a zeta-zeta homodimeric structure or a zeta-eta heterodimeric structure in addition to these five other components. This group has used gene transfection studies to try to address the subunit interactions within the receptor complex. In addition, this group has focused on the zeta and eta chains of the TCR complex, both in terms of the molecular biology, the biochemistry, the assembly and structure/function relations. Eta deficient variants have been isolated and characterized, as well as zeta negative variants and mutants. The eta chain has been biochemically characterized and its immunologic relationship to zeta identified. The zeta chain is transcriptionally regulated in a very intriguing way, and this has been correlated with structural changes in the gene. The full structure of the gene has been identified. The structure of zeta has been altered by site-directed mutagenesis and this has been utilized to reconstruct the first signal transduction mutants of the TCR complex.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01606-01 CBMB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Biology of Early Organelles of the Secretory Pathway

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	R.D. Klausner	Head	CBMB, NICHD
Others:	J.S. Bonifacino	Visiting Associate	CBMB, NICHD
	J. Lippincott-Schwartz	NRSA	CBMB, NICHD
	L.C. Yuan	Technician (Chem.)	CBMB, NICHD
	C. Suzuki	Adjunct Technician	CBMB, NICHD
	J.G. Nuchtern	IRTA Fellow	CBMB, NICHD

COOPERATING UNITS *(if any)*
Neuroimmunology Branch, NINCDS (W.E. Biddison)

LAB/BRANCH
Cell Biology and Metabolism Branch

SECTION
Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	4	1

CHECK APPROPRIATE BOX(ES)
- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This group has been engaged in studying the structure, function and dynamics of the early organelles of the secretory vacuolar system. The group studies three aspects of these problems: 1) Architectural editing - a concept that has evolved out of the work of this group in explaining the cell biologic processes that underlie the ability of the cell to correctly structure newly synthesized membrane proteins and to edit out incomplete or incorrect proteins or protein complexes. One of the major mechanisms by which the cell prevents the expression on the cell surface of abnormal membrane proteins is by targeting those proteins for degradation. This group has identified a new pathway for intracellular degradation that takes place within the endoplasmic reticulum. 2) The structure and dynamics of membrane flow in the secretory pathway - this group has identified a novel drug, Brefeldin A, that has unique effects on organellar structure and dynamics. Based upon the effects of this drug we have characterized new pathways of movement between the Golgi, intermediate compartments, and the endoplasmic reticulum. 3) Antigen presentation - this group has identified the endoplasmic reticulum as the major site for the presentation of endogenous antigens to major histocompatability complex molecules.

DEVELOPMENTAL ENDOCRINOLOGY BRANCH (DEB)

ZO1 HD 00610-09	Puberty and its Disorders: Physiology, Pathophysiology and Therapy Gordon B. Cutler, Jr., M.D.
Z01 HD 00615-09	Steroid Antagonists George P. Chrousos, M.D.
Z01 HD 00616-09	Structure, Function, and Physiology of Glycoprotein Hormones Bruce C. Nisula, M.D.
Z01 HD 00618-08	Physiology and Pathophysiology of the Hypothalamic-Pituitary-Adrenal Axis George B. Chrousos, M.D.
Z01 HD 00619-08	Hypothalamic Pituitary Gonadal Interactions D. Lynn Loriaux, M.D.
Z01 HD 00621-07	Mechanism of Linear Growth Fernando Cassorla, M.D.
Z01 HO 00622-07	Diagnostic and Therapeutic Applications of Growth Hormone-Releasing Hormones George R. Merriam, M.D.
Z01 HD 00623-06	Adrenal Physiology and Pathophysiology Gordon B. Cutler, Jr., M.D.
ZO1 HD 00625-02	Neuroendocrine Regulation of Reproductive Function George R. Merriam, M.D.
ZO1 HD 00626-01	Progesterone Action in Reproduction Lynette Nieman, M.D.

Developmental Endocrinology Branch

The Developmental Endocrinology Branch conducts basic and clinical investigations of endocrine diseases. Major areas within the multifaceted research program are devoted to adult, pediatric, and reproductive endocrine topics. Within each area, the Branch employs a broad array of fundamental biochemical, pharmacological, and physiological approaches with the aims of promoting knowledge of endocrine diseases and developing new diagnostic and therapeutic techniques. Thus, a major objective in the Branch's mission in endocrinology is the translation of basic biomedical research findings into practical bedside applications through clinical research. The spectrum of endocrine disorders under study consists of those affecting the testis, ovary, adrenal, thyroid, pituitary, and hypothalamus. Of particular interest are factors regulating and diseases affecting the pituitary-adrenal, pituitary-gonadal, and pituitary-thyroid axes.

The Section on Developmental Endocrinology, led by **Gordon Cutler, Jr.**, studies the basic mechanisms and clinical disorders of growth, puberty, and adrenal function. The objective of these studies is to gain new insights into basic molecular mechanisms, physiology, and pathophysiology that can be applied to the prevention, diagnosis, and treatment of endocrine diseases. The methods employed range from the basic approaches of molecular endocrinology to controlled clinical trials. A major strategy of the section is to employ the techniques of molecular endocrinology to gain insight into the identity and the relative importance of the growth factors and other substances that regulate epiphyseal growth.

Three major clinical trials are in progress to evaluate new approaches to the treatment of short stature. The first tests the hypothesis that prolonging the growth period by delaying puberty can enhance adult height. The observation that patients with pubertal delay due to isolated hypogonadotropic hypogonadism are significantly taller than normal subjects provides support for this hypothesis. The second clinical trial tests the hypothesis that supplemental growth hormone, with or without low dose ethinyl estradiol, will increase the adult height of girls with Turner syndrome. Since girls with Turner syndrome average more than 3 SD below the normal mean height (4' 9"), short stature is the major concern of patients with this disorder. The outcome variables include data on both safety and efficacy, and will provide a scientific basis for rational decision regarding the risk to benefit ratio of this new treatment option. The third clinical trial tests the hypothesis that supplemental growth hormone will increase the adult height of children with non-growth hormone-deficient short stature (constitutional, familial, or idiopathic short stature). This trial will meet an important societal need for objective outcome data upon which to make rational risk-benefit analyses concerning this new treatment approach. The number of short children in the United States who are potential candidates for growth hormone treatment is 1,000,000. Thus, the answers sought by this trial have important clinical and regulatory as well as scientific significance.

A major unresolved issue in pediatric endocrinology has been how best to establish, in a scientifically valid manner, the diagnosis of growth hormone (GH) deficiency. A recent study from this section, published in the New England Journal of Medicine, showed that the mean 24-hour GH level has much lower sensitivity for the diagnosis of GH deficiency than had been previously reported. This has rekindled interest in GH stimulation tests as the most efficient and reliable means of diagnosing GH deficiency.

The molecular mechanisms involved in puberty are an area of active interest within the section. During the current year, the human LHRH gene has been cloned and the structure of its regulatory sequences determined. Estrogen regulates the gene both positively (5' flanking sequences only) and negatively (entire gene). The basis for the divergent effects of estrogen in different portions of the gene is presently under investigation. The positive regulation of the gene by estrogen has been localized to a cognate estrogen response element at about 450 base pairs upstream from the major transcription start site. Future studies will examine the regulatory effects of other steroid hormones and of thyroid hormone.

The differential diagnosis of delayed puberty continues to pose a difficult clinical problem for the endocrinologist. No definitive means exists currently to distinguish the child who has constitutional delay of puberty, and therefore will enter puberty spontaneously, from the child who has isolated hypogondotropic hypogonadism and will require hormonal treatment to induce puberty. Recent studies in the prepubertal rhesus monkey have shown that pulsatile administration of N-methyl-D-aspartate (NMDA) can trigger pulsatile release of LHRH and puberty. This suggested that it may be feasible to develop a functional test of the integrity of the LHRH neuron based upon stimulation of the NMDA receptor. To test this concept, the naturally occurring ligands of the NMDA receptor, aspartate and glutamate, were administered to prepubertal rhesus monkeys whose gonadotroph cells had been rendered responsive to LHRH through a brief period of pulsatile LHRH pretreatment. Both aspartate and glutamate induced a brisk response of both LH and FSH at the dose of 150 mg/kg intravenously. Future studies will examine the feasibility of a clinical test of LHRH neuron function based upon these observations. The hypothesis is that such a test would reveal integrity of the LHRH neuron in children with constitutional delay of puberty, and show deficient LHRH neuron responsiveness in isolated hypogonadotropic hypogonadism.

Studies of early puberty have led to recent advances in both pathophysiology and treatment. The insight into pathophysiology is the demonstration of LH-like bioactivity in the sera of boys with gonadotropin-independent familial male precocious puberty (FMPP). Current research is focussing on the chemical structure of this LH-like bioactivity.

A new approach to the treatment of FMPP has proven effective in normalizing the rate of growth and bone maturation and in preventing acne, spontaneous erections, and aggressive behavior. The treatment combines an antiandrogen (spironolactone) to block androgen action with an inhibitor of androgen-to-estrogen conversion (testolactone) to prevent estrogen effects on epiphyseal growth. Current therapeutic research is directed at evaluating the long-term effects of this new treatment, at improving treatment with more specific and/or potent antiandrogens and aromatase inhibitors, and at treating secondary central precocious puberty, when necessary, by administration of LHRH analog as well.

Ongoing studies of adrenal physiology and pathophysiology have the objective of improving the treatment of congenital adrenal hyperplasia and of improving the diagnosis, differential diagnosis, and treatment of Cushing's syndrome. One clinical trial involving congenital adrenal hyperplasia employs a combination of antiandrogen and aromatase inhibitor, which is analogous to the strategy that was employed successfully in familial male precocious puberty. It is anticipated that blockade of androgen action and of estrogen synthesis will normalize growth and bone maturation, and thus permit hydrocortisone dosage to be reduced to strictly physiologic levels.

The overnight dexamethasone suppression test is employed widely as a screening test for Cushing's syndrome and as a diagnostic test for depression. Heretofore, it has not been known whether the feedback effects of dexamethasone are exerted primarily at the hypothalamus to decrease CRH release, or at the pituitary, to inhibit ACTH synthesis and release. To dissect these potential sites of negative feedback, basal and insulin-induced ACTH and cortisol levels were examined on a control day, after the overnight dexamethasone suppression test, and after overnight dexamethasone followed by pulsatile hCRH administration. Dexamethasone inhibited both basal and hypoglycemia-induced cortisol and ACTH levels. Pulsatile hCRH, administered after dexamethasone, failed to prevent suppression of basal cortisol but did prevent suppression of the cortisol response to hypoglycemia. These results are consistent with inhibition by dexamethasone at both the hypothalamic and pituitary levels. At the hypothalamic level, overnight dexamethasone administration inhibited basal pituitary-adrenal function but not the response to insulin-induced hypoglycemia. At the level of the corticotroph, overnight dexamethasone inhibited the response to hypoglycemia, however, this inhibition was prevented by pulsatile hCRH. Thus, pituitary inhibition in the overnight dexamethasone suppression test appears to result primarily from the loss of hypothalamic CRH priming rather than from a direct effect of dexamethasone on the pituitary.

Despite recent advances in the differential diagnosis of Cushing's syndrome, the distinction between Cushing's disease (ACTH-secreting pituitary microadenoma) and ectopic ACTH-secreting neoplasms remains a difficult problem. Several advances in this area have been made in the past year. First, the sensitivity of magnetic resonance (MR) scanning for pituitary microadenomas has been improved by doing the procedure with and without the contrast agent gadolinium. A single scan is not optimal because of the heterogenous nature of the pituitary lesions (e.g., solid, cystic, etc.). Some lesions are revealed by gadolinium, whereas others that are visible without contrast are obscured by gadolinium. Optimal diagnosis thus requires both types of scan. Second, MR scanning was found to be superior to computed tomography (CT) for the detection of ACTH-secreting bronchial carcinoid tumors within the hilar region of the lung. In this region tumors can be mistaken for blood vessels by CT, whereas MR distinguishes tumor from blood vessels, which fail to image by MR because of movement of blood in the interval before signal generation. Thus, it was found that optimal examination of the lung for ectopic ACTH-secreting tumors requires MR for the hilar region (inner 1/3) and CT for the outer 2/3, where the higher speed of CT imaging results in better resolution. In addition, increased resolution of both MR and CT scanning have resulted in improved diagnosis of micronodular adrenal disease. Although the micronodules in this condition have been regarded as too small to be imaged by radiographic procedures, it has recently proven possibly to identify nodules in approximately 90% of these patients, with good correlation between the radiographically identified nodules and the nodules identified pathologically after adrenalectomy. Since the normal adrenal size in patients with micronodular adrenal disease can suggest the possibility of factitious Cushing's syndrome, the ability to diagnose this disorder radiographically will help resolve a difficult diagnostic problem.

A careful retrospective analysis of patients who have had second or third transsphenoidal explorations for Cushing's disease has provided new guidance concerning when to consider this treatment approach for recurrent Cushing's disease. Although the overall remission rate was 60%, the results were considerably poorer in patients whose initial exploration revealed tumors adjacent to the lateral walls of the cavernous sinus. Patients who underwent an inadequate initial exploration (e.g., patients in whom a sphenoid sinus septum was left intact or in whom the window in the sellar floor was small) or who had more centrally located tumors at initial exploration, had considerably better results at second exploration. Thus, based on these studies, repeat transsphenoidal surgery is no longer recommended for patients with recurrent Cushing's disease in whom the probability of tumor extension into the walls of the cavernous sinus appears high.

A major limitation in the postoperative recovery of patients with Cushing's disease who have been cured by transsphenoidal surgery is secondary adrenal insufficiency, which can require up to a year to resolve. To distinguish whether this adrenal insufficiency results primarily from suppression of the CRH neuron by longstanding hypercortisolism or from a direct effect of hypercortisolism on the pituitary, pulsatile human CRH was administered for one week to patients who had been cured of Cushing's disease. Pulsatile CRH administration had no effect on recovery of the hypothalamic-pituitary adrenal axis. The response to CRH, even after one week of pulsatile administration, remained far below control levels. These observations indicated that the secondary adrenal insufficiency of patients cured of Cushing's disease is associated with a profound unresponsiveness of the corticotroph cell to CRH that cannot be corrected by one week of pulsatile CRH administration. However, this finding does not exclude the possibility of a coexisting suppression at the level of the CRH neuron. Taken together, the above work during the previous year has contributed significantly to improving the accuracy of diagnosis and the effectiveness of treatment of Cushing's syndrome, a potentially fatal endocrine disorder that often strikes children or young adults.

The Medical Endocrinology Section, directed by **Bruce Nisula**, conducts research on the glycoprotein hormones, chorionic gonadotropic (hCG), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH). During the past year, this Section has performed research on a number of topics, including pregnancy loss in healthy women, developmental characteristics of the thyroid axes in childhood, structural determinants of the pharmacokinetic properties of glycoprotein hormones and functional significance of carbohydrate modifications with respect to glycoprotein hormone action in vivo.

A large population of healthy couples was studied to define the fundamental fertility characteristics of the normal population. Prior to these studies, it had been known that healthy couples having intercourse regularly without contraception have about a 25 percent chance of achieving a pregnancy in a menstrual cycle. Of these recognized pregnancies, a percentage are lost as spontaneous abortions. A spontaneous abortion is a loss of pregnancy that has been clinically recognized by the woman and her physician. The goal of the study was to define, in addition, how often women lose pregnancies that are unrecognized by them. Such early pregnancy losses would usually be inapparent to the women because they did not miss their menstrual period or the pregnancies occurred and were lost before the expected menses. Samples and data were collected over a total of 707 menstrual cycles from 221 healthy women attempting pregnancy. Each pregnancy was identified by measurement of hCG, a hormone made by the developing placenta. The criterion for identifying a pregnancy was an hCG level exceeding 0.025 ng/ml for three consecutive days; a group of 28 women who had

undergone sterilization by tubal ligation were consistently below this level. Of 198 pregnancies, 80 percent had detectable elevations in hCG levels by the 28th day and 50 percent by the 25th day of the menstrual cycle. Thus, measurement of hCG in urine allowed detection of pregnancy very close to the time of implantation and prior to recognition of the pregnancy by the women (i.e., before the delay of menses occurred). The total rate of pregnancy loss after implantation was 31 percent. Early pregnancy losses (22 percent of all pregnancies) were far more common than spontaneous abortions (9 percent of all pregnancies). Thus, health women conceive, progress to the implantation stage, and lose many more pregnancies than are clinically apparent. The data on these women were analyzed to evaluate the implication of a pregnancy loss for future fertility. Heretofore, it had been clinical practice to advise women that pregnancy loss contributes to infertility. In fact, the opposite tendency applied. Of the women who had an early pregnancy loss, 35 percent became pregnant in the very next cycle, 68 percent with three cycles, and 83 percent within six cycles. By comparison, in the general population, 25 percent became pregnant in one cycle and 74 percent within six menstrual cycles. These data show not only that early pregnancy loss is a normal phenomenon in healthy women attempting pregnancy, but also that early pregnancy loss tends to imply enhanced future fertility, rather than infertility. Such findings provide important impetus for modifying current clinical practice, which has been to advise women to use birth-control methods for a while after a pregnancy loss. These findings indicate that postponement of pregnancy loss is an unnecessary practice, and in the older woman, it may even be detrimental.

Growth and development in children is critically dependent on normal thyroid function. Diagnosis and management of thyroid diseases in children have heretofore been hampered by a lack of critical information on the normal development of pituitary-thyroid axis function. Information in the literature was either non-existent, incomplete, or controversial. Accordingly, the pituitary-thyroid axis function of 96 normal children, aged 5-18 years, was investigated. The circadian variation of serum TSH was examined in relation to chronological age, and its relationships to serum levels of T4, T3, and thyroxine-binding globulin (TBG) were determined. Children at all ages exhibited a circadian pattern of serum TSH concentration, characterized by a nadir in the late afternoon, a rise to a peak around midnight, a plateau for several hours, and then a decline in the daytime. The mean nadir TSH was 1.6 ± 0.1 in U/L, and the mean peak TSH was 3.7 ± 0.2 U/L. The mean nocturnal TSH surge (percent increase in TSH from nadir to peak) was 144 percent. Neither TSH concentration nor the nocturnal TSH surge correlated significantly with age or serum thyroid hormone levels. In contrast, serum T4, T3, and TBG exhibited a significant negative correlation with age. These findings are consistent with the interpretation that developmental changes in serum thyroid hormones in children largely reflect changes in hormone transport by TBG, and are not regulated by changes in TSH secretion. This study establishes, for the first time, the normal developmental pattern of TSH in childhood, and shows that normal children have a circadian variation of serum TSH quite similar to that of adults. Thus, physicians caring for children with thyroid diseases and disorders affecting thyroid function now have available to them systematic, detailed TSH information on which to base their medical judgments. In addition to providing normative data on development, these data contribute important new evidence concerning the mechanism of the developmental changes in thyroid hormone levels in childhood. The findings point to alterations in hormone transport, rather than changes in TSH secretion as the underlying mechanism. Also of physiological interest in this investigation was the observation that the magnitude of the nocturnal TSH peak correlated significantly with the peak serum TSH after TRH infusion. Some workers have postulated that the nocturnal surge of TSH is the principal component of TSH secretion that is regulated by increased secretion of TRH by the hypothalamus. These

data lend credence to this possibility.

The structure-kinetic relationships of hCG and related molecules were investigated in the current year to assess how profound differences in carbohydrate and/or polypeptide structures affect parameters of plasma turnover of glycoprotein hormones. The impetus to the study derived in part from a controversy, recently raised in the literature, over whether the carbohydrate structure of a glycoprotein hormone is a determinant of the initial volume of distribution. Theoretical considerations would dictate that the initial volume of distribution would correspond to the intravascular plasma space (volume). To address this question, the pharmacokinetics of native hCG, desialylated hCG (ds-hCG), deglycosylated hCG (dg-hCG), and the core fragment of hCGβ (β-core) were analyzed in male cynomolgus monkeys. The metabolic clearance rates of dg-hCG, β-core, and ds-hCG were increased 15-, 47-, and 152-fold, respectively, over that of hCG. However, despite these huge differences in kinetic behavior, the initial volumes of distribution were indistinguishable among them: hCG (0.035 ± 0.002 L/kg), ds-hCG (0.034 ± 0.004); ds-hCG (0.035 ± 0.004); and β-core (0.035 ± 0.002). The ds-hCG is devoid of its terminal sialic acid and has its terminal galactose exposed. The dg-hCG has lost about 70 percent of its carbohydrate residues, retaining for the most part its most proximal N-acetyl-D-glucosamine and N-acetyl-D-galactosamine. The β-core retains the trimannosyl core of the asparagine-linked carbohydrate and lacks the antennary sugar residues as well as the O-linked carbohydrate chains. Thus, as theory would predict, dramatic variations in polypeptide and carbohydrate composition do not affect the initial volume of distribution, but rather dictate profound changes in the rate constants of disappearance. These latter parameters are presumed to reflect cellular uptake processes localized mainly to the liver, kidney, and reticuloendothelial system. These findings indicate that discrepancies in work published by others are artifactual and related either to methodological issues or purity of the glycoprotein preparations employed. In addition to resolving an issue fundamental to glycoprotein hormone physiology, these studies lay the groundwork for the development of analogs with desirable pharmacokinetic characteristics for clinical applications.

Previous structure-function studies had indicated that intact carbohydrate structures are essential for the full expression of the biological effects of glycoprotein hormones. For example, removal of the carbohydrate residues from hCG (deglycosylation) drastically reduced the in vitro rodent gonadal cAMP response to the hormone analog; and removal of the terminal sialic acid residues from hCG (desialylation) accelerated plasma clearance and thereby reduced potency in rodent in vivo bioassays. In the current period, the consequences of these carbohydrate modification on the intrinsic biological activity of hCG in a primate model, the male cynomolgus monkey, was studied. Large doses of highly purified dg-hCG, ds-hCG, hCG, or normal saline were administered and the plasma testosterone response measured. Interestingly, the mean plasma testosterone responses over the first 6 hours achieved with dg-hCG, ds-hCG, and hCG were indistinguishable despite the fact that the mean concentrations differed dramatically among the groups. Plasma ds-hCG and dg-hCG were undetectable by 15 and 180 min, respectively, while the mean plasma hCG was more than 2.1 nmol/L at 360 min. While the in vivo testosterone response to dg-hCG was indistinguishable from that to hCG, their in vitro adenylate cyclase responses differed dramatically. The dg-hCG preparation had less than 4 percent of the maximal agonist activity of hCG and, in fact, was able to antagonize hCG-stimulated adenylate cyclase to approximately 6 percent of the maximal hCG-stimulated level. Thus, dg-hCG elicited a full testosterone secretory response in the primate in vivo, despite having minimal intrinsic activity and despite being a powerful antagonist of hCG action on adenylate cyclase. These findings have illuminated an important attribute of the signal transduction system in the testis: Powerful antagonists of hCG action on adenylate cyclase, such as dg-hCG,

are paradoxically capable of stimulating a full testosterone secretory response. The concept of superfluity in cAMP production, which characterizes the testicular Leydig cell signal transduction system, explains the seeming paradox. Thus, a minuscule amount of the intermediary messenger cAMP is sufficient to evoke a maximal and sustained testosterone secretory response as the bulk of the cAMP-producing capacity inherent in the Leydig cell is superfluous as far as the secretory response is concerned. Earlier studies by others established the spare receptor phenomenon; more recent studies with analogs of glycoprotein hormones, such as dg-hCG, have called attention to the superfluous cAMP phenomenon and enhanced our understanding of signal transduction mechanisms.

The Section on Pediatric Endocrinology, headed by **George Chrousos**, investigates the hypothalamic-pituitary-adrenal (HPA) axis from physiological and pathophysiological perspectives. During the past year, the antiglucocorticoid compound RU 486 and the recently discovered corticotropin-releasing hormone (CRH) were used to elucidate the fundamental functional characteristics of the HPA axis under various physiological conditions, under physical and emotional stress, and in major endocrine diseases and psychiatric disorders.

In dose-response studies with the antiglucocorticoid RU 486 in nonhuman primates and humans, sustained elevations of plasma ACTH, cortisol, and arginine-vasopressin were observed. RU 486 bound strongly to plasma proteins, yielding a relatively long plasma half-life of about 20 h in humans. Less than 1% of the administered RU 486 was excreted in urine. This knowledge of the pharmacokinetics of RU 486 was key to devising therapeutic regimens. Treatment of several patients suffering from profound hypercortisolism due to the ectopic ACTH syndrome or adrenal carcinoma with RU 486 resulted in dramatic remissions from their clinical manifestations. Somewhat paradoxically, RU 486 was able to suppress the concentration of ACTH in the plasma of patients with adrenal insufficiency given test doses of CRH. While this result suggested some intrinsic glucocorticoid activity in RU 486, that activity was insufficient to sustain cardiovascular homeostasis in adrenalectomized monkeys. Thus, RU 486 approaches being a pure competitive antagonist at the glucocorticoid receptor. As such, it was used to address two longstanding questions about the effects of glucocorticoids on the immune system: (1) Are the immunological effects of glucocorticoids receptor-mediated? and (2) Do glucocorticoids play a role in modulating the immune system at basal, resting physiological levels? In an "aseptic inflammation" rat model in which glucocorticoids suppress exudate formation, leukocyte diapedesis, and prostanoid accumulation, RU 486 was able to antagonize the effect of pharmacologic doses of glucocorticoids, suggesting that anti-inflammatory effects are indeed receptor-mediated. In addition, RU 486 given alone caused enhancement of the inflammatory response, suggesting that endogenous glucocorticoids at physiological levels exert suppressive effects on the inflammatory response in the rat. RU 486 also caused enlargement of the thymus and spleen in these animals. These observations suggested that a glucocorticoid antagonist could be used as an immune enhancer in conditions that would benefit from such enhancement (cancer, immunodeficiency). This concept was explored in part in normal volunteers receiving RU 486 daily. The excretion of urinary free cortisol was increased markedly in these volunteers but there was no detectable change in various circulating lymphocyte markers. Similarly, there were no detectable changes in the ability of the lymphocytes to become activated and to become more cytotoxic. These findings suggest that glucocorticoid effects on the immune system are not exerted in the basal state in primates, a significant difference from rodents. In other studies, RU 486 was instrumental in identifying a central nervous system defect in rats prone to arthritis in response to antigenic stimulation with streptococcal cell wall polysaccharide. These rats (Lewis rats) had deficient CRH,

ACTH and glucocorticoid responses to stressors. A finding of particular interest was that histocompatible rats (Fisher rats) which normally do not develop arthritis in response to streptococcal cell wall polysaccharide did so when treated with RU 486.
The work outlined above has established that RU 486 carries promise as a diagnostic and therapeutic tool and as a probe to study adrenal and gonadal physiology. As a challenger of the hypothalamic-pituitary-adrenal axis, it offers a key tool in the study of disorders of the axis: Cushing's syndrome, adrenal insufficiency, psychiatric hypercortisolism. As a therapeutic tool it appears to be useful in treating a subset of patients with hypercortisolism. As an experimental tool, it provides an excellent means to probe stress physiology and the role of glucocorticoids upon the immune system and the inflammatory response.

Hypothalamic corticotropin-releasing hormone (CRH) and the glucocorticoid receptor are the main regulators of the HPA axis. The recent discovery of CRH has given great impetus to stress research and to the study of pediatric psychiatric disease. In addition, its availability has revised the way we evaluate and treat the major diseases of the HPA axis, including Cushing's syndrome (hypercortisolism), and adrenal insufficiency. Dr. Chrousos and his colleagues have pioneered in the application of CRH in the differential diagnosis of major endocrine diseases, in elucidating pathophysiological mechanisms in major endocrine and psychiatric disorders, and in exploring its role in responses to physical and emotional stress. With respect to clinical diagnostic applications, the CRH stimulation test has significantly improved our capacity to differentiate the causes of Cushing's syndrome and, hence, to treat successfully the various forms of this entity early in their course. The CRH test also proved to be an excellent means of differentiating between the primary, secondary, and tertiary forms of adrenal insufficiency. Moreover, in patients with hypercortisolemic psychiatric disease, the CRH test and measurement of CRH in the cerebrospinal fluid suggested that a major pathophysiologic feature of these states-- hyperfunction of the CRH neuronal system. This finding was particularly important because CRH appears to be a major biochemical coordinator of the stress response; hence, it probably not only stimulates ACTH and glucocorticoid secretion, but also activates the central sympathetic and arousal systems, and produces characteristic behavioral and biochemical changes consistent with chronic stress and the syndrome of depression. As an example, the central administration of CRH resulted in anorexia, decreased libido, hypothalamic hypogonadism, and anxiety, all of which are among the cardinal manifestations of major depression. In addition, CRH given intracerebroventricularly to experimental animals produced limbic seizures that cross-sensitized with electrically-kindled seizures. This finding may be relevant to the natural history of recurrent affective disorder, which is characterized by a gradual increase in the severity and frequency of episodes throughout its course. As a corollary, it has been known since the mid-sixties that infant experimental animals that are separated from their mothers for prolonged periods of time show an accentuation of the hypothalamic-pituitary-adrenal responsiveness to stress throughout life. Interestingly, they also show increased vulnerability to the sensitization of key limbic sites induced by CRH.

Studies of the regulation of hypothalamic CRH secretion in both <u>in vivo</u> and <u>in vitro</u> models have yielded new insights. They have demonstrated that the hypothalamic CRH neuron is under the stimulatory influence of serotonergic, cholinergic, norepinephrinergic, dopaminergic, epinephrinergic and histaminergic neurons and the inhibitory influence of GABA/benzodiazepine and opioid peptide systems. The CRH neuron appears also to be regulated by a series of negative feedback loops: a CRH-mediated (ultrashort) loop, an ACTH- and beta-endorphin-mediated (short) loop and a glucocorticoid-mediated (long) loop. The fact that norepinephrine stimulates CRH secretion settled a long-standing controversy; moreover, this finding not only provides

a putative mechanism for the concordance in the activation of the sympathetic nervous system and the HPA axis during stress, but also provided a more firm rationale for the mechanism of action of many antidepressant and anxiolytic medications.

Studies of the interaction between the immune and CRH systems have also yielded novel findings in the past year. Several cytokines, including interleukin I, tumor necrosis factor-alpha, as well as several lipid mediators of inflammation, caused profound stimulation of the HPA axis via activation of the CRH neuron. Thus, it appears that the CRH neuron provides a link between the immune and central nervous systems by which cytokines/lipids stimulate CRH-mediated glucocorticoid-induced negative feedback of the inflammatory/immune response. Bearing importantly on the concept of a link between the immune system and the HPA axis were results of studies of the interesting animal model discussed above, in which autoimmune arthritis is associated with a poor CRH response to inflammatory mediators and subsequent hyper-responsiveness of the immune system. This suggests a novel mechanism for the pathogenesis of autoimmune disease.

The HPA axis plays an integral role in the normal physiology of pregnancy, labor, and delivery. Current studies have shown that the placenta secretes CRH as well as ACTH and beta-endorphin. The production of these hormones was shown to increase gradually in the third trimester of pregnancy and then increase further in labor. It appears that there is a paracrine/autocrine regulatory loop in the placenta with CRH stimulating local secretion of ACTH and beta-endorphin. Further, the HPA axis may be involved in the immunosuppression known to occur in pregnancy and in the development of the post-partum depression syndromes, both of which are under study.

The Section on Steroid Hormones, led by **D. Lynn Loriaux**, has as its objective the furthering of our understanding of the role of steroid hormones in the complex processes of growth, development, and reproduction. A corollary of this is to understand the diseases associated with these processes and, where possible, to initiate and to evaluate rational therapy for them. The problems currently being addressed include delineation of the mechanisms of glucocorticoid resistance, development of novel methods for quantitation of steroid secretion in various diseases, and elucidation of the role of progesterone in the menstrual cycle and in the regulation of specific endometrial proteins.

The glucocorticoid receptors from two models of glucocorticoid resistance have been studied. The gene encoding the glucocorticoid receptor from the New World Primate has been cloned and sequenced. Multiple base differences were evident when a New World species gene was compared with the Old World species gene. In particular, changes were evident in the steroid binding region and in the region that is thought to bind heat shock protein, potentially an important cofactor in glucocorticoid action. This was not found when the receptor from a common human resistant state, Cushing's Syndrome, was examined. Preliminary studies of size and RFLP patterns revealed no differences between the receptors from these tumors and receptors from adjacent normal tissue. Studies in the coming year will focus on identifying the mutation in the New World Primate that is responsible for the glucocorticoid resistance by synthesizing glucocorticoid receptors with promising mutations and examining their action using a standard reporter gene technique.

Studies of the cortisol production rate using a deuterated trace cortisol molecule in normal subjects and patients with Cushing's Syndrome have demonstrated complete separation of the two groups and have provided a benchmark for the evaluation of the standard tests employed for the diagnosis of Cushing's Syndrome. An unexpected result

was the finding that the levels of 17-hydroxy steroids correlate better with the cortisol production rate than does the urinary free cortisol concentration.

Studies in a primate model of depression have focused on elucidating the mechanism of increased cortisol production in these animals. Preliminary studies by this group have attempted to clarify whether or not the pituitary ACTH secretion characteristics of the disorder is CRF dependent. Inferior petrosal sinus sampling has revealed that CRF is easily measurable in these animals, suggesting that the hypercortisolism of affective disease is hypothalamic in origin.

Progesterone is essential for the normal morphologic development of the luteal phase endometrium, for implantation of a fertilized egg and for maintenance of pregnancy. Surprisingly, given its critical role in reproduction, little is known about the mediator(s) of progesterone action. In the current year, a recently described progestin-dependent glycoprotein, placental protein 14 (PP14), and the antiprogestin RU 486 have been used to probe progesterone action. The relationship between endogenous progesterone production, serum PP14 concentrations and endometrial maturation was associated with low PP14 and progesterone values. Further studies in infertile women and those with multiple miscarriages should give better insight into the cause, and possible, reveal a path to the treatment of these disorders.

Guinea pigs, like women, require progesterone for normal endometrial development, implantation and pregnancy. The antiprogestin RU 486 was employed to probe the process of implantation in this animal model. Small daily doses of the drug resulted in a marked decrease in implantation sites, confirming the critical role of progesterone in nidation. Studies in non-pregnant women not at risk for pregnancy showed that small daily doses of RU 486 delay the normal maturation of the endometrium without significantly changing the hormonal patterns or timing of the menstrual cycle. No adverse effects were seen in either study. Treatment with RU 486 is a model for corpus luteum deficiency which holds promise for helping elucidate the way(s) in which this condition results in infertility.

The Unit on Linear Growth Physiology, directed by **Fernando Cassorla**, investigates the hormonal mechanisms that are responsible for linear growth. Principal areas of investigation in the current year include improving the accuracy of the methods employed to diagnose growth hormone deficiency, studying the effects of growth hormone and sex steroid administration on linear growth in patients with delayed puberty, and determining the cortisol production rate in normal children and adolescents. In other studies, pubertal delay is being induced in children with extreme short stature with the objective being to prolong prepubertal growth prior to the pubertal spurt and possibly enhance ultimate height by delaying epiphyseal fusion. The impact of growth hormone therapy on the adult height of non-growth-hormone deficient children with short stature is being investigated through a randomized, double-blind, placebo-controlled clinical trial. Finally, the effect of growth hormone-releasing factor on linear growth in growth hormone-deficient children is being determined under different treatment regimens in order to optimize growth.

The daily cortisol production rate in normal children and adolescents was determined using a stable isotope-dilution technique employing high-performance liquid chromatography-mass spectrometry (LC/MS). Thirteen normal subjects, ages 8-16 years, were studied. Deuterated cortisol was infused continuously for 30 hours at 5% of the anticipated daily cortisol production rate. After 6 hours of tracer infusion, which were needed to achieve steady-state levels, blood was obtained every 20 min for 24 hrs. Deuterated cortisol dilution in plasma was determined by LC/MS. The cortisol

production rate was 9.8 ± 2.7 mg/day (mean ± SD) in the normals and did not vary with sex. The cortisol production rate was 10.1 ± 2.9 mg/M^2/day in 7 prepubertal children, and 10.0 ± 3.0 mg/day in 4 Tanner V adolescents. Corrected for surface area, the cortisol production rate was 8.5 ± 2.3 mg/M^2/day in the Tanner V adolescents ($p<0.05$). These results suggest that the cortisol production rate in children and adolescents is significantly lower than previously estimated. The implication of these data is that the replacement doses of glucocorticoids currently used in the treatment of adrenal insufficiency need to be carefully reevaluated. When patients are replaced with glucocorticoids at the currently accepted dose of 12 mg/M^2/day, signs of glucocorticoid excess may develop. The cortisol production rate observed in this study of normal children and adolescents was considerably lower than the currently recommended replacement and, thus, suggests that many children with adrenal insufficiency have been given excessive doses of glucocorticoids heretofore. This has profound implications for the management of children with adrenal insufficiency, whether associated with panhypopituitarism or congenital adrenal hyperplasia. It is expected that prescribing more physiologic replacement doses of glucocorticoids will allow these patient to grow better and to avoid developing evidence of glucocorticoid excess.

Boys with isolated hypogonadotropic hypogonadism and those with anencephaly may show microphallus and subnormal testicular growth at birth. The failure to virilize fully has been attributed to the lack of gonadotropic or testosterone stimulation in the latter part of gestation. In the current year, a primate model of this human condition was devised by suppressing pituitary-gonadal activity with a long-acting LHRH analog ($LHRH_A$) from mid-gestation through early infancy in male cynomolgus monkeys. The effects of this suppression on sexual development and growth were carefully monitored. Thus, D-Trp^6-Pro^9-NEt-LHRH in microspheres or placebo were injected subcutaneously into fetal cynomolgus monkeys, and the external genitalia, weight, linear growth, and pituitary-gonadal function of the 2 groups were compared. Plasma FSH, LH and testosterone levels were lower in the $LHRH_A$-treated group than in the placebo group as expected. The striking finding was that the testicular and phallic size in the $LHRH_A$-treated group were significantly less than in the placebo group. The results clearly show that suppression of the hypothalamic-pituitary-gonadal axis in-utero leads to impaired phallic and testicular growth during early infancy in cynomolgus monkeys. This model shows that gonadal stimulation by the fetal pituitary during the latter half of gestation is responsible for penile and testicular growth. Dr. Cassorla and his colleagues are now determining whether this pituitary-gonadal activation in-utero has any long-term fertility effects by studying the pubertal development and reproductive function of these animals. This work will help us to understand the significance of pituitary-gonadal activation in utero and the consequences of its derangement such as that observed in patients with hypogonadotropic hypogonadism.

The Unit on Reproductive Endocrinology, directed by **George Merriam**, investigates the regulation of reproductive function, and the neuroendocrine control of gonadotropins and of growth hormone. Studies in reproduction emphasize the neuroendocrine control of the normal menstrual cycle, and the disorders of hypothalamic amenorrhea, hypogonadism, luteal phase deficiency, premature ovarian failure, and ovarian hyperandrogenism.

In the normal menstrual cycle, previous studies by this group have shown that a small rise in progesterone precedes the ovulatory midcycle LH surge, and may be the ovarian signal that the dominant follicle is ready to be ovulated. To determine if this is so, normally cycling women received 1 mg of the progesterone antagonist RU486 or placebo daily in the late follicular phase of the cycle. Although ovarian follicular development

continued unimpeded, ovulation was delayed by 3-4 days in the treated group. When the experiment was repeated in 5 women with hypothalamic amenorrhea treated with pulsatile GnRH, a similar delay was observed, indicating that hypothalamic effects are unnecessary to the action of progesterone, which therefore probably occurs at the pituitary level. When supplemental progesterone was given along with RU486, the delay inovulation was reversed, confirming the specificity of the effect. Thus, progesterone secretion may well serve as an important regulatory signal.

Seasonal changes in reproductive function dominate the regulation of breeding in many species, but the role of photoperiod in the control of reproduction in man and higher primates is unclear. A series of studies in rhesus monkeys housed under controlled environmental conditions and artificial lighting has demonstrated for the first time that photoperiod is also a potent regulator of reproduction in primates. When the lighting pattern was switched from short to long days (16 hours of light per day), prolactin levels and gonadal function were suppressed; the switch from long to short days (8 hours of light) reversed this and reactivated gonadal function. Further, when the photoperiod was held constant, without periodic adjustments, rhythmic changes in reproductive status still occurred, suggesting the presence of an endogenous circannual rhythm. This is the longest endogenous periodicity to be demonstrated in higher animals.

The suppression of cyclic menses during lactation is one of the major factors affecting the spacing of children in most of the world; yet, the mechanism of this effect is poorly understood. Effects at CNS, pituitary, and gonadal levels have all been postulated. Initially levels of prolactin are elevated, and a parallel has been drawn to the amenorrhea of prolactinoma patients. Subsequently, prolactin falls, but amenorrhea persists as long as suckling continues. Studies under way are aimed at elucidating the mechanism of postpartum amenorrhea. The gonadotropin pattern in these subjects showed a marked suppression of pulsatile gonadotropin secretion during lactational amenorrhea, with recovery as soon as suckling ceased. Treatment with pulsatile GnRH during lactation resulted in normal follicular development, ovulation, and a normal luteal phase, essentially ruling out an ovarian abnormality. Thus lactational amenorrhea appears to be an entirely neuroendocrine phenomenon. Companion studies in animals are examining the changes in mRNA for gonadotropin-releasing hormone during lactational hypogonadism, using <u>in situ</u> hybridization histochemistry, to determine if the altered function is regulated at this level.

Luteal phase dysfunction is a poorly understood syndrome in which it has been proposed that inefficient endometrial maturation accounts for a failure of normal implantation. This could be due to insufficient progesterone secretion by the corpus luteum, or an inadequate endometrial response. The diagnosis of luteal phase dysfunction is uncertain, and is currently based upon endometrial biopsies, which are painful and not well calibrated. Previous work by this group has shown that progesterone secretion in the luteal phase is pulsatile; thus, single measurements of progesterone can be very misleading, and measuring integrated progesterone secretion is not practical for clinical purposes. Since luteal function remains under LH control, in principle the response to a standardized gonadotropin challenge could provide a better calibrated measure of corpus luteum status. Studies now under way show that administration of hCG can provide a reliable stimulus to progesterone secretion, with less variability than that obtained from single measurements, and that responses are low in patients with abnormal endometrial biopsies. If confirmed, these studies could lead to a non-invasive test of luteal function for use in the evaluation of infertile women.

Menopause normally occurs after age 50, and a cessation of menses before age 40 is termed premature ovarian failure. Generally this condition is considered to represent an irreversible depletion of ovarian follicles, like normal menopause. However, this is not always so, and remissions of ovarian failure with resumption of ovulation have been reported, occurring either spontaneously or after estrogen replacement. Autoimmune ovarian disease has been reported as a cause of premature ovarian failure, and it is likely that may patients with idiopathic ovarian failure also have autoimmune oophoritis. The association of remission with estrogen therapy suggests that not all follicles are destroyed, but only those stimulated to grow by gonadotropins. This makes it credible to hypothesize that gonadotropin stimulation leads to expression of the antigens which are targets of the autoimmune process. By removing gonadotropin stimulation, one might reduce the antigenic burden and allow a temporary remission to occur. Several studies are underway to test this hypothesis in patients and animals and to study the mechanisms involved.

Hyperandrogenism is a common cause of oligomenorrhea and infertility, but still poses etiologic, diagnostic, and therapeutic challenges. An association of polycystic ovary syndrome with insulin resistance or obesity suggests a link with hyperinsulinism, perhaps by action at the IGF-1 receptor, but this is unproven. A whole-follicle ovarian organ culture technique has been developed to allow studies of the effects of changes in IGF-1 and other growth factors on thecal transformation of ovarian stromal fibroblasts. Study of ovarian function and morphology in patients with acromegaly and high IGF-1 levels showed a high incidence of polycystic ovaries, consistent with the hypothesis that IGF-1 is indeed involved in the control of androgen production.

Another major area of interest of this group is the neuroendocrine control of growth hormone (GH), and the diagnostic and therapeutic applications of growth hormone-releasing hormone (GHRH). Pituitary GH is essential for normal growth, and is important in the normal regulation of metabolism. GHRH and somatostatin (SRIF) are the two hypothalamic peptides which together regulate GH snythesis and secretion. A principal aim is to explore the role of GHRH and related peptides in the treatment of GH deficiency and GH excess. Early studies from this group showed that intensive treatment with multiple daily doses of GHRH can stimulate GH secretion and restore growth in most children with GH deficiency. This demonstrates that in most cases GH deficiency, in fact, represents a hypothalamic deficiency of GHRH, and that the pituitary can be stimulated to produce sufficient quantities of GH to support normal growth. These intensive regimens are sufficiently complex to be impractical by comparison with conventional GH treatment regimens. Current studies have now demonstrated that even a single daily dose of GHRH can restore normal growth in these patients, a regimen which is as simple and more physiologic than conventional treatment with GH. It also appears possible that co-administration of a drug which suppresses SRIF, which blocks GH secretion, may enhance the therapeutic response to GHRH and further improve growth.

GHRH, the physiologic stimulator of GH release, is a 44-amino acid peptide. A hexapeptide derived from enkephalin and unrelated to GHRH has been shown to have potent GH-releasing activity in primates and man, and can stimulate a much greater rise in GH than can GHRH itself. Its dose-response curve however, is not parallel to that of GHRH, indicating a different mechanism of action. The mechanism of action and potential clinical utility of this peptide are both under study. Its action at the pituitary level differs from that of GHRH, with much less stimulation of cyclic AMP. It appears that in addition to pituitary effects, the hexapeptide stimulates the release of GHRH from the hypothalamus in vitro, and its effects in animals are blocked by inhibitors of GHRH secretion. This dual effect may account for its high potency in

vivo and its differences from GHRH.

Overproduction of GH is most commonly due to pituitary tumors and can cause crippling deformities. Conventional therapy of these tumors with surgery, radiation, or drugs is frequently unsuccessful. Work in this group has shown that these tumors remain sufficiently well differentiated to respond to GHRH with a rise in cyclic AMP and GH. When the tumors cannot be visualized in patients using conventional imaging techniques, measuring GH in blood sampled from both inferior petrosal sinuses can indicate on which side the tumor is located, facilitating tumor removal. Progress has also been made on a novel approach to therapy of these tumors. GHRH is linked to a toxin which would then be concentrated in tumor cells, allowing them to be killed. An organoboron jugate of GHRH as been synthesized which emits lethal but very localized alpha radiation upon exposure to neutrons, and has been found to be biologically active and concentrated in pituitary cells. Its ability to induce cytotoxicity in pituitary cells is being tested.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00610-09 DEB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Puberty and its Disorders: Physiology, Pathophysiology and Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: G.B. Cutler, Jr. Head, DES, DEB, NICHD

Others: (see attached list)

COOPERATING UNITS *(if any)*

See Attached

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Developmental Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.4	4.5	0.9

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to advance understanding of the mechanisms that underlie normal and abnormal puberty, and to apply this knowledge to improve existing therapy for disorders of puberty. Since somatic growth is a major determinant of the timing of pubertal onset, a further objective is to clarify the mechanisms of normal growth and of growth failure. Principal areas of clinical investigation include mechanism of premature thelarche and of the gonadotropin-independent forms of precocious puberty, the developmental changes in hypothalamic regulation of gonadotropin secretion, the behavioral changes associated with normal and abnormal pubertal development, the mechanisms of prepubertal and pubertal growth, the role of pubertal sex steroids in the acquisition of normal adult bone density, the treatment of central precocious puberty with an analog of luteinizing hormone-releasing hormone, the treatment of familial male isosexual precocious puberty with combined antiandrogen and aromatase inhibitor, the evaluation of new approaches to the diagnosis of growth hormone deficiency and to the differential diagnosis of delayed puberty, and the treatment with growth hormone of children with Turner syndrome and with non-growth hormone-deficient short stature.

The principal areas of laboratory investigation include the structure and function of the regulatory sequences of the human gonadotropin-releasing hormone (GnRH) gene, the structure of the human GnRH gene in families with GnRH-dependent precocious puberty or with idiopathic hypogonadotropic hypogonadism, linkage analysis of the gene for familial male precocious puberty to the luteinizing hormone receptor or other loci, and hormonal regulation of epiphyseal transforming growth factor ß and fibroblast growth factor.

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Cooperating Units

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00615-09 DEB

PERIOD COVERED: October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Steroid Antagonists

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

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COOPERATING UNITS (if any): Clinical Neuroendocrinology Branch, NIMH, NIH (P.W. Gold, E. Sternberg); Surgery Branch, NIH (J. Norton); Clinical Pathology Department, Clinical Center, NIH (T. Fleisher)

LAB/BRANCH: Developmental Endocrinology Branch

SECTION: Unit on Clinical Neuroendocrinology

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	2.0	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

Clinically useful antagonists exist for estrogens, androgens, and mineralocorticoids. Antagonists for the glucocorticoids or the progestins with potential clinical usefulness have been discovered only recently. The objective of this project is to develop and study the molecular mechanisms of action and the human applications of the antagonists for both of these classes of steroids. We have tested a prototype glucocorticoid-progestin antagonist (RU 486) developed recently by Roussel-UCLAF. This compound has strong affinities for the human glucocorticoid and progestin receptor and is devoid of agonist effects in small experimental animals.

Given to nonhuman primates or man RU 486 causes prolonged elevations of plasma ACTH, cortisol and arginine vasopressin, all changes preventable by previous administration of a glucocorticoid (dexamethasone). This suggests that antiglucocorticoids could be used for challenging the hypothalamic-pituitary-adrenal axis, when clinical testing is required in patients with disorders of this axis. Antiglucocorticoid therapy of patients with severe Cushing's syndrome due to ectopic ACTH secretion or adrenocortical tumors causes remission of the clinical manifestations of hypercortisolism. We have treated several patients and are currently enlarging the therapy series.

RU 486 allowed the identification of a central nervous system defect in rats prone to arthritis. In these animals the glucocorticoid response to stress-mediators is inadequate to restrain the immune system following an insult. This pathophysiologic mechanism is novel and its relevance to human arthritis will be examined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00616-09 DEB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure, Function, and Physiology of Glycoprotein Hormones

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B.C. Nisula	Head, SME	DEB, NICHD
Others:	D. Blithe	Sr. Staff Fellow	DEB, NICHD
	R. Wehmann	Special Expert	DEB, NICHD
	M. Flack	Med. Staff Fellow	DEB, NICHD
	C. Lyons	Bio. Lab. Tech.	DEB, NICHD

COOPERATING UNITS *(if any)*
Epidemiology Branch, NIEHS (A.J. Wilcox, C.R. Weinberg, and D.D. Baird)

LAB/BRANCH
Developmental Endocrinology Branch

SECTION
Medical Endocrinology Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The general goal of this project is to understand the structure, function, and physiology of the human glycoprotein hormones, thyroid-stimulating hormone (TSH), choriogonadotropin (hCG), luteinizing hormone (LH), and follicle-stimulating hormone (FSH), and thereby to develop diagnostic and therapeutic clinical applications. Recent research advances include the following: Demonstration that in healthy women attempting pregnancy the total rate of pregnancy loss is 31 percent, with more than two-thirds of these being lost before being clinically recognized by the women or their physicians; observation of enhanced fertility, rather than infertility in women following an early pregnancy loss occurrence; characterization of the developmental pattern of the pituitary-thyroid axis and the circadian pattern of TSH in normal children; and elucidation of structure-function relationships of hCG and related molecules in the determination of pharmacokinetic parameters and in vivo gonadal responses. Future studies under this project will explore the mechanisms and significance of the structural heterogeneity of glycoprotein hormones that occurs in health and in various diseases. Investigation of structure-function relationships through molecular biological techniques such as site-directed mutagenesis will be undertaken; the focus will be on the role of specific carbohydrate side chains in subunit combination, hormone secretion, and biological efficacy of hormone product. In addition, the fundamental physiological parameters of beta-core metabolism in humans will be determined in order to quantify the placental secretion of beta-core and the placental contribution to overall production of the molecule in pregnancy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00618-08 DEB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Physiology and Pathophysiology of the Hypothalamic-Pituitary-Adrenal Axis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: G.P. Chrousos Head UCN, DEB, NICHD

Others: (see attached list)

COOPERATING UNITS *(if any)*

(see attached list)

LAB/BRANCH
Developmental Endocrinology Branch

SECTION
Unit on Clinical Neuroendocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.1	6.1	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In this project we seek to advance the understanding of the physiology and pathophysiology of the hypothalamic-pituitary-adrenal axis. The role of stress-related hormones in normal and disease states is being examined, and clinical applications for these hormones are sought. The recent discovery of the structure of corticotropin releasing hormone (CRH) and the development of sensitive assays for measuring stress-related hormones and their receptors have led to rapid progress in this field. Major progress has been made in three areas:

1) Clinical applications of CRH: An ovine CRH (oCRH) stimulation test has been developed that is useful in the differential diagnosis of adrenal insufficiency, Cushing's syndrome, and pseudo-Cushing's states (psychiatric hypercortisolism). The human CRH (hCRH) analog is useful in studying the physiology of the pituitary-adrenal axis. The oCRH stimulation test and measurement of CSF CRH have increased our understanding of the pathophysiology of Cushing's syndrome, depression, anorexia nervosa, the chronic fatigue syndrome and late-onset congenital adrenal hyperplasia.

2) The regulation of the axis by opioids, vasopressin, oxytocin, glucocorticoids, platelet activating factor (PAF), tumor necrosis factor - alpha (TNF-alpha), cholecystokinin (CCK), neuropeptide Y and benzodiazepine agonists and antagonists has been studied in vivo and/or in vitro. Neurotransmitter and feedback regulation of hypothalamic CRH secretion has also been examined in vitro. Athletes have a hyperfunctional pituitary-adrenal axis in the resting state. Hypothalamic-pituitary-adrenal axis reactivity and personality traits have been correlated in developing adolescents.

3) Role and actions of glucocorticoids: The effects of glucocorticoids upon the cardiovascular system during surgical stress are merely permissive. Glucocorticoid resistance is associated with normal size glucocorticoid receptor protein that has decreased affinity for glucocorticoids and normal size mRNA.

Others:	R. Bernardini	Adjunct Scientist	DEB, NICHD
	J. Brockmann	Adjunct Scientist	DEB, NICHD
	A. Calogero	Adjunct Scientist	DEB, NICHD
	P. Feuillan	Adjunct Scientist	DEB, NICHD
	W. Gallucci	Adjunct Technician	DEB, NICHD
	T. Gomez	Clinical Associate	DEB, NICHD
	D. Hurley	Adjunct Scientist	DEB, NICHD
	T. Kamilaris	Adjunct Scientist	DEB, NICHD
	S. Listwak	Adjunct Technician	DEB, NICHD
	A. Margioris	Adjunct Scientist	DEB, NICHD
	E. McClure	Adjunct Scientist	DEB, NICHD
	L. Nieman	Expert	DEB, NICHD
	D. Rabin	Adjunct Scientist	DEB, NICHD
	N. Vamvakopoulos	Adjunct Scientist	DEB, NICHD

Cooperating Units

Clinical Neuroendocrinology Branch, National Institute of Mental Health, NIH (P.W. Gold); Surgical Neurology Branch, National Institute of Neurological and Communicative Disorders and Stroke, NIH (E. Oldfield); Laboratory of Developmental Psychology, National Institute of Mental Health, NIH (E. Nottleman); Human Performance Laboratory, Dept of Military Medicine, USUHS (P. Deuster).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00619-08 DEB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Hypothalamic Pituitary Gonadal Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.L. Loriaux	Head	SSH, DEB, NICHD
Others:	(see attached list)		

COOPERATING UNITS *(if any)*
Roussel - UCLAF, Paris, France (E.E. Baulieu)

LAB/BRANCH
Developmental Endocrinology Branch

SECTION
Section on Steroid Hormones

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS.	PROFESSIONAL:	OTHER:
4.0	4.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Work in the past year has focused on the mechanism of action of the glucocorticoids, the mechanisms of glucocorticoid resistance, the quantitation of glucocorticoid secretion and metabolism in normal and abnormal states, and the mechanisms underlying abnormal 'activation' of the hypothalamic-pituitary-adrenal axis. Results from the years work have shown that glucocorticoid resistance in the New World Primate is associated with mutations in the glucocorticoid receptor in both the steroid binding region and in the region believed to bind heat shock protein. The glucocorticoid resistance found in pituitary tumors secreting ACTH is not associated with any observable mutation in the glucocorticoid receptor. Studies of cortisol production rate in normal subjects and patients with Cushing's Syndrome have demonstrated complete separation of the two groups and have provided a benchmark for the evaluation of the standard tests employed for the diagnosis of Cushing's Syndrome. Finally, a model of depression in a primate has been under study to elucidate the mechanism underlying activation of the hypothalamic-pituitary-adrenal axis in this disorder. Early results suggest that CRF is necessary for this activation; other requirements are under continued study.

Others:	D. Brandon	Sr. Staff Fellow	DEB, NICHD
	B. Albertson	IPA	DEB, NICHD
	L. Laue	IPA	DEB, NICHD
	J. Zawadski	IPA	DEB, NICHD
	M. Batista	Fogarty Visiting Fellow	DEB, NICHD
	M. Flores	Fogarty Visiting Fellow	DEB, NICHD
	T. Wheler	Medical Staff Fellow	DEB, NICHD
	N. Saliba	Adjunct Scientist	DEB, NICHD
	R. Weber	Adjunct Scientist	DEB, NICHD

Cooperating Units

Steve Soumi, LCE, NICHD
Bill Stokes, SMRA, OSD, NICHD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00621-07 DEB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Linear Growth

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: F. Cassorla Sr. Investigator DEB, NICHD

Others: (see attached)

COOPERATING UNITS *(if any)* Metabolism Branch, NCI, NIH (P. Nissley); Molecular, Cellular & Nutrition Endocrinology Branch, NIDDK, NIH (S. Radovick); Laboratory of Cellular Development & Oncology, NIDR, NIH (A.H. Ridde); Hahnemann Medical School, Philadelphia, Pennsylvania (J. Levine)

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Unit on Growth Physiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	2.5	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to investigate the hormonal mechanisms that are responsible for linear growth. Principal areas of investigation include improving the accuracy of the methods employed to diagnose growth hormone deficiency. We are also studying the effects of growth hormone and sex steroid administration on linear growth in patients with Turner's syndrome and delayed puberty. We are also determining the cortisol production rate in normal children and adolescents. In addition, we are studying the mechanism of catch up growth in a primate model. We are also examining the effect of inducing pubertal delay in children with extreme short stature, in order to prolong prepubertal growth prior to the pubertal spurt and possibly enhance ultimate height by delaying epiphyseal fusion. We are also investigating the effects of growth hormone therapy on the adult height of non-growth-hormone deficient children with short stature through a randomized, double-blind, placebo-controlled clinical trial. In addition, we are investigating the growth hormone secretory dynamics in patients with hypophosphatemic rickets. Finally, we are studying the effects of growth hormone-releasing factor on linear growth in growth hormone-deficient children by using different treatment regimens in order to optimize growth.

Others:

B. Linder	Medical Staff Fellow	DEB, NICHD
A. Cristiano	IRTA	DEB, NICHD
G. Marin	Visiting Fellow	DEB, NICHD
S. Rose	Adjunct Scientist	DEB, NICHD
S. Malozowski	Adjunct Scientist	DEB, NICHD
J. Weissman	Adjunct Scientist	DEB, NICHD
G. Municchi	Adjunct Scientist	DEB, NICHD
G.B. Cutler	Head, SDE	DEB, NICHD
G.R. Merriam	Head, UCN	DEB, NICHD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00622-07 DEB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diagnostic and Therapeutic Applications of Growth Hormone-Releasing Hormones

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: G.R. Merriam Head URE, NICHD

Others: (see attached list)

COOPERATING UNITS *(if any)*

(see attached list)

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Unit on Reproductive Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.3	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The pituitary hormone growth hormone (GH) is essential for normal growth and is important in the normal regulation of metabolism. Growth hormone-releasing hormone (GHRH) and somatostatin (SRIF) are the two hypothalamic peptides which together regulate GH synthesis and secretion. This project aims to explore the role of GHRH and related peptides in the treatment of GH deficiency and GH excess; to study the neuroendocrine regulation of GH secretion; and to attempt to define abnormalities which may contribute to the etiology of GH-secreting and the pituitary tumors. Treatment of GH deficiency: We have previously shown that intensive treatment with multiple daily doses of GHRH can stimulate GH secretion and restore growth in most children with GH deficiency. In a study now in progress, we find that even a single daily dose of GHRH can restore a normal growth rate in these patients, a regimen which is as simple and more physiologic than conventional treatment with GH. We are studying whether co-administration of a drug (atenolol) to suppress SRIF, which blocks GH secretion, can enhance the therapeutic response to GHRH and further improve growth.

Evaluation and therapy of acromegaly. Conventional therapy of pituitary tumors which produce GH (acromegaly) is frequently unsuccessful. Since these tumors have receptors for GHRH, we are testing whether GHRH can be linked to a toxin which will then be concentrated in tumor cells, allowing them to be killed. We have synthesized an organoboron conjugate of GHRH which emits lethal but very localized alpha radiation on exposure to neutrons, and have shown that it is biologically active and localized in pituitary cells. We are currently testing its ability to enhance neutron cytotoxicity in rat pituitary cells. In a separate project, we have shown that when acromegalic tumors cannot be visualized in patients using conventional imagining techniques, measuring GH in blood sampled angiographically from both inferior petrosal sinuses can indicate on which side the tumor is located, facilitating surgical removal.

Others:

F. Cassorla	Head, ULGP	DEB, NICHD
D.L. Loriaux	Head, SSH	DEB, NICHD
M. Flack	Med. Staff Fellow	DEB, NICHD
E.H. Hao	Visiting Fellow	DEB, NICHD
A. Cristiano	NRSA Fellow	DEB, NICHD
T. Loughlin	Visiting Fellow	DEB, NICHD
A. Calogero	Visiting Fellow	DEB, NICHD
N. Ma	Adjunct Scientist	DEB, NICHD
S. Malozowski	Adjunct Scientist	DEB, NICHD
S. Rose	Adjunct Scientist	DEB, NICHD

Cooperating Units: ERRB, NICHD (H.C. Chen); HGB, NICHD (W. Gahl); Surg. Neurol. Br, NINDS (E. Oldfield); CBMB, NICHD (S. Frank); State Univ. N.Y., Stony Brook (M.C. Gelato); Univ. Indiana (O. Pescovitz); Univ. Catania (R. D'Agata). Dept. Diag. Radiol, CC, NIH (J. Doppman); Univ. Calif., L.A. (F. Hawthrone); LMB, NCI (I. Pastan); National Institute of Standards and Technology (E. Carter).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00623-06 DEB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Adrenal Physiology and Pathophysiology

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	G.B. Cutler, Jr.	Head	DES, DEB, NICHD
Others:	(see attached)		

COOPERATING UNITS *(if any)*
(see attached list)

LAB/BRANCH
Developmental Endocrinology Branch

SECTION
Section on Developmental Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER.
3.2	3.1	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We seek to advance understanding of the mechanisms that cause adrenal androgen secretion by the fetal adrenal zone prenatally and by the definitive adrenal cortex during adrenarche, and to improve the diagnosis and treatment of disorders that cause excess adrenal androgen or glucocorticoid secretion, such as premature adrenarche, congenital adrenal hyperplasia, adrenal neoplasms, idiopathic hirsutism, polycystic ovary syndrome, and Cushing's syndrome. We also seek to clarify the pathophysiology of primary adrenal insufficiency (Addison's disease) and secondary adrenal insufficiency and to improve the treatment of these conditions.

Children with congenital adrenal hyperplasia are being enrolled into one of three projects. The first examines the effect of several recent advances in treatment on long-term growth and adult height. The second tests the effect of different dose-schedules of hydrocortisone. The third employs a newly available antiandrogen and an inhibitor of androgen-to-estrogen conversion to block the action of excess androgen levels.

Patients with Cushing's syndrome are being studied by several new diagnostic methods to determine the relative diagnostic efficiency of these new methods compared to the old. Patients with Cushing's syndrome due to occult ACTH-producing tumors are being treated with an investigational glucocorticoid antagonist. Oncogene expression in corticotropinomas is also under study in an attempt to elucidate the molecular basis of Cushing's disease.

Others:	D.L.Loriaux	Chief	SSH, DEB, NICHD
	B. Albertson	Adjunct Scientist	Georgetown University
	K.M. Barnes	Chemist (Tech)	DEB, NICHD
	F. Cassorla	Head, ULGP	DEB, NICHD
	G. Chrousos	Head, UHRF	DEB, NICHD
	P. Feuillan	Adjunct Scientist	Eli Lilly and Company
	L. Laue	Med. Staff Fellow	DEB, NICHD
	T. Loughlin	Visiting Fellow	DEB, NICHD
	L. Nieman	Expert	Roussel-UCLAF
	S. Radovick	Senior Staff Fellow	MCNEB, NIDDK
	J. Levine Ross	Adjunct Scientist	Hahnemann University
	H. Tracer	Medical Staff Fellow	DEB, NICHD

Cooperating Units

Chief, Radiology, CC, NIH (J. Doppman); Acting Chief, SNB, NINCDS, NIH (E. Oldfield); Staff Radiologist, Radiology, CC, NIH (A.J. Dwyer); Department of Internal Medicine, McMaster University Medical Center (J. Booth); Senior Staff Fellow, MCNEB, NIDDK (S. Radovick, F. Wondisford).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00625-02 DEB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neuroendocrine Regulation of Reproductive Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G.R. Merriam	Head	URE, DEB, NICHD
Others:	(see attached list)		

COOPERATING UNITS *(if any)*
(see attached list)

LAB/BRANCH
Developmental Endocrinology Branch

SECTION
Section on Steroid Hormones

INSTITUTE AND LOCATION
NICHD, NIH Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.3	8.0	0.3

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project aims to clarify some of the mechanisms which control reproduction, and to study new approaches to the evaluation and treatment of disorders of reproductive function in men and women. The emphasis is on the neuroendocrine control of normal reproduction, and the disorders of hypothalamic amenorrhea and hypogonadism; luteal phase deficiency; premature ovarian failure; and ovarian hyperandrogenism. Reproductive Physiology: In the normal menstrual cycle, a small rise in progesterone precedes the midcycle surge, and may be the signal that the dominant follicle is ready for ovulation. A progesterone antagonist administered to subjects in the preovulatory period significantly delays ovulation, although ovarian function continues normally. This indicates that progesterone is an important regulator of the timing of ovulation. Hypothalamic amenorrhea: In studies of normal postpartum subjects, we have found that pulsatile gonadotropin secretion is inhibited during lactational amenorrhea, suggesting a central etiology to this disorder. Further, when GnRH is given by pulsatile infusion, we find that normal ovarian function is restored, essentially ruling out an ovarian abnormality. Premature ovarian failure (POF): Cessation of menses before age 40 is a cause of infertility whose causes are poorly characterized. There have been reports of POF in association with autoimmune failure of other organs. Some patients with POF experience remissions, indicating that not all the ovarian follicles are destroyed, We are testing the hypothesis that an autoimmune process may only attack developing follicles, and that a period of ovarian suppression with GnRH analog may lead to a remission. Ovarian hyperandrogenism/polycystic ovary syndrome (PCOS): PCOS is a common cause of anovulation as well as a source of considerable morbidity due to androgen excess. It has been speculated that hyperinsulinism may cause this disorder. We are testing the hypotheses that insulin is in part acting by activation of receptors for the insulin-like growth factor IGF-1, and that either an excess of insulin/IGF-1 or a hypersensitivity to these factors results in an increase of the steroidogenic enzymes 17-hydroxylase and 20,22-desmolase, leading to excessive androgen production.

Others:	D.L. Loriaux	Head	SSH, DEB, NICHD
	L.K. Nieman	Expert	SSH, DEB, NICHD
	R. Hertz	Scientist Emeritus	DEB, NICHD
	M.C. Batista	Visiting Fellow	DEB, NICHD
	H.C.L. Bohler	NRSA Fellow	DEB, NICHD
	L.M. Nelson	NRSA Fellow	DEB, NICHD
	M. Zinaman	IPA	DEB, NICHD
	A. Armstrong	Adjunct Scientist	DEB, NICHD
	R. Levy-Toledano	Adjunct Scientist	DEB, NICHD
	L. Liu	Adjunct Scientist	DEB, NICHD
	J. Lopaczynska	Adjunct Scientist	DEB, NICHD

Cooperating Units: Laboratory of Pathology and Medicine Branch, NCI, NIH (M. Merino, A. Kruisbeek); Clinical Center, NIH (T. Fleisher, R. Kimzey, T. Cartledge); NIMH, NIH (M. Mishkin, D. Rubinow, P. Schmidt); Max-Planck Institut (O.F.X. Almeida); Yale University (J. Luborsky); University of Texas (E. Simpson); Mt. Sinai School of Medicine (A. Dunaif); Salk Institute (W. Vale); University of Melbourne (H. Burger); MRC Reproductive Biology Unit (G. Lincoln).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00626-01 DEB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Progesterone Action in Reproduction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.K. Nieman	Expert	DEB, NICHD
Other:	M. Batista	Visiting Fellow	DEB, NICHD
	D.L. Loriaux	Head	SSH, DEB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Developmental Endocrinology Branch

SECTION
Section on Steroid Hormones

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS.	PROFESSIONAL:	OTHER:
1.8	1.8	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Progesterone is essential for the normal morphologic development of the luteal phase endometrium, for implantation of a fertilized egg and for maintenance of pregnancy. Surprisingly, given its critical role in reproduction, little is known about the mediator(s) of progesterone action. We are using a recently described progestin-dependent glycoprotein, placental protein 14 (PP14), and the antiprogestin RU 486 to probe progesterone action. We are investigating the relationship between endogenous progesterone production, serum PP14 concentrations and endometrial maturation in women. These studies suggest that PP14 concentrations reflect previous progesterone levels, and that a delay in endometrial maturation is associated with low PP14 and progesterone values. Further studies in infertile women and those with multiple miscarriages should give us better insight into the cause and, possibly, the treatment of these disorders.

The guinea pig, like women, requires progesterone for normal endometrial development, implantation and pregnancy. We have used the antiprogestin RU 486 to probe the process of implantation in this animal model. Small daily doses of the drug result in a marked decrease in implantation sites, confirming the critical role of progesterone in nidation. Studies in non-pregnant women not at risk for pregnancy show that small daily doses of RU 486 delay the normal maturation of the endometrium without significantly changing the hormonal patterns or timing of the menstrual cycle. No adverse effects were seen in either study. This work suggests that RU 486 has potential as a contraceptive agent.

ENDOCRINOLOGY AND REPRODUCTION RESEARCH BRANCH (ERRB)

Z01 HD 00022-16	Renin-Angiotensin System and Aldosterone Regulation Greti Aguilera, M.D.
Z01 HD 00035-17	The Structure and Function of Biologically Active Molecules Hao-Chia Chen, Ph.D.
Z01 HD 00146-14	The Structure and Function of Chorionic Gonadotropins Hao-Chia Chen, Ph.D.
Z01 HD 00147-14	Mechanism of Action of Peptide Hormones in Steroidogenic Cells Maria L. Dufau, M.D., Ph.D.
Z01 HD 00149-14	Bioassay of Serum Luteinizing Hormone (LH) and Chorionic Gonadotropin Maria L. Dufau, M.D., Ph.D.
Z01 HD 00150-14	Characterization of Gonadal Prolactin, LH/hCG Receptors and Coupling Functions Maria L. Dufau, M.D., Ph.D.
Z01 HD 00151-14	Regulation of Gonadal and Placental Function Kevin J. Catt, M.D., Ph.D.
Z01 HD 00184-11	Regulation of Pituitary Hormone Secretion Kevin J. Catt, M.D., Ph.D.
Z01 HD 00187-10	Hormonal Regulation of Cellular Metabolism Kuo-Ping Huang, Ph.D.
Z01 HD 00191-05	Mechanisms of Neuroendocrine Regulation Greti Aguilera, M.D.
Z01 HD 00193-04	Angiotensin II Receptors and Activation Mechanisms Kevin J. Catt, M.D., Ph.D.
ZO1 HD 00194-01	ACTH Regulation of Adrenocortical Function (Steroidogenesis) Biochemistry and Molecular Biology Charles A. Strott, M.D.

Endocrinology and Reproduction Research Branch

The research programs of the Endocrinology and Reproduction Research Branch are directed at the elucidation of cellular mechanisms involved in hormone secretion and action. These programs include studies on the characterization of peptide hormones and their cellular receptors; the mechanisms of peptide hormone action in endocrine target cells; the structure-function relationships of peptide and glycoprotein hormones; the regulation of hormone biosynthesis and secretion. Of particular interest are the investigation of pituitary-gonadal and pituitary-adrenal regulation, the control of ovarian activity during the reproductive cycle and pregnancy, and the receptor-mediated control of pituitary, gonadal, and adrenal function. During the current year, research has been performed on the receptors and signalling processes that are responsible for the control of secretory responses and differentiation in endocrine target cells. The role of hormones in cellular regulation has also been examined in selected forms of normal and disordered human endocrine function, and in appropriate animal model systems for the analysis of hormone secretion and the stimulatory and inhibitory control of target-cell function. The staff of the ERRB share common interests in the mechanisms of action of peptide and glycoprotein hormones, the role of neuropeptides in hypothalamic-pituitary regulation and stress, the control of gonadal and adrenal function by pituitary hormones, the renin-angiotensin system and aldosterone secretion, and the mechanisms and roles of protein phosphorylation in metabolic regulation. The major research programs of the Branch are supervised by the respective senior investigators under the following organizational units within the ERRB:

The Section on Hormonal Regulation (Dr. Kevin Catt) performs research on the control of endocrine target cells by peptide hormones, in particular the charac-terization, regulation, and activation mechanisms of membrane receptors for gonadotropin-releasing hormone (GnRH), angiotensin II (AII), and gonadotropins. The receptor-mediated actions of hypothalamic releasing peptides and other regulators of pituitary hormone secretion are studied in cultured anterior pituitary cells. The actions of angiotensin II are investigated in cultured adrenal glomerulosa cells, and those of gonadotropins are analyzed in ovarian granulosa and luteal cells.

Pituitary gonadotropin secretion is primarily regulated by GnRH, which binds to and activates specific receptors in the plasma membrane of the gonadotroph. The GnRH receptor has been solubilized in active form with non-ionic detergents and shown to exist as a 250 kDa complex under non-denaturing conditions. This large complex contains a 52 kDa component that is demonstrable by photo-affinity labeling, and which corresponds to the GnRH binding subunit of the receptor. GnRH action in pituitary gonadotrophs is expressed through the activation of calcium-phosphoinositide signaling mechanisms, leading to a characteristic biphasic profile of cytosolic calcium elevation and gonadotropin release from the pituitary gland. The initial phase of LH secretion has been found to depend largely on mobilization of intracellular calcium, whereas the sustained phase of hormone secretion is dependent on continued entry of extracellular calcium through plasma-membrane channels. In addition to the $InsP_3$-mediated early rise in [Ca]i and LH release, other phospholipid products including diacylglycerol (DG) and arachidonic acid (AA) are involved in cellular activation by GnRH. Studies with enzyme inhibitors revealed that GnRH activates two distinct lipases, DG lipase and

phospholipase A_2, to produce arachidonic acid, which is metabolized to HETEs and leukotrienes in pituitary cells. Activation of protein kinase C by DG also participates in GnRH action, in part by mechanisms independent of calcium mobilization, but also by promoting calcium influx through dihydropyridine-insensitive plasma-membrane channels. The involvement of protein kinase C in gonadotropin synthesis and secretion was also shown by studies in pituitary cells depleted of protein kinase C, in which the secretory actions of GnRH and phorbol esters were substantially impaired. These findings indicate that protein kinase C participates in both the acute and long-term regulatory actions of GnRH in pituitary gonadotrophs, by enhancing calcium entry during the secretory response to agonist stimulation and also by increasing gonadotropin synthesis during prolonged exposure to GnRH.

Studies on the nature of the calcium channels involved in pituitary hormone secretion revealed the presence of L-type voltage sensitive calcium channels (VSCC) in all five populations of anterior pituitary cells, with an average number of about 1000 channels per cell. In gonadotrophs, calcium entry through L-type VSCC was found to account for about 50% of the elevated cytosolic calcium and LH secretory response during sustained stimulation by GnRH. Furthermore, marked inactivation of VSCC during GnRH action was shown to occur by a calcium-dependent mechanism and to be accompanied by a corresponding decrease in LH responsiveness to GnRH. This process of calcium channel inactivation is responsible for the onset of desensitization, a prominent feature of GnRH action and the cellular basis of therapeutic suppression of gonadotropin secretion by treatment with GnRH superagonists.

In addition the well-recognized form of hypothalamic regulation by GnRH, gonadotrophs were found to be more slowly activated by neurohypophyseal hormones. This action was shown to be mediated by a distinct receptor of the oxytocin type, in contrast to the vasopressin-type receptor that has been shown to mediate ACTH release in pituitary corticotrophs. This action of oxytocin could provide a complementary hypothalamic control mechanism for the long-term modulation of gonadotropin secretion, and could contribute to the basal level of LH secretion upon which the pulsatile action of GnRH is superimposed to produce the normal physiological profile of episodic gonadotropin release.

The structural properties and activation mechanisms of the angiotensin II (AII) receptor were studied in bovine adrenal glomerulosa cells and other target tissues. The adrenal AII receptor was purified by affinity chromatography and subjected to microsequencing, and cDNA probes were prepared and applied to the screening of an adrenal cDNA library. A further approach to cloning the AII receptor was initiated by screening mRNA, prepared from an adrenal cDNA expression library cloned into phage lambda Zap, in the *Xenopus* oocyte. The detection systems employed in this approach were based on the measurement of electrophysiological and light responses (measured by the photoprotein, aequorin) in oocytes injected with mRNA from adrenal extracts and the expression library. The aequorin detection system was established and validated by measurement of the light responses elicited by ligand-induced calcium mobilization in *Xenopus* oocytes injected with poly $(A)^+$ mRNA from rat adrenal cortex and brain, during stimulation by AII, acetylcholine and glutamate. This system in currently under application to the detection of angiotensin II receptor mRNA in the phage expression library, and to the analysis of the properties of endogenous and exogenous AII receptors in the oocyte.

Studies on the mechanism of action of angiotensin II, performed in cultured bovine adrenal glomerulosa cells, showed that the rapid effect of AII on Ins-1,4,5-P_3 formation was accompanied by a concomitant elevation of cytosolic calcium during the first minute of stimulation, with peaks in both $InsP_3$ and $[Ca^{2+}]i$ responses at 5-10 sec. The subsequent plateau in calcium was associated with secondary increases in Ins-1,4,5-P_3 and Ins-1,3,4,5-P_4, suggesting that both inositol phosphates could participate in the maintenance of the sustained calcium response to agonist stimulation. Extending the Section's previous studies on the production and metabolism of inositol phosphates in AII-stimulated adrenal glomerulosa cells, the conversion of Ins-1,3,4,6-P_4 inositol pentakisphosphate ($InsP_5$) was demonstrated in bovine adrenal cytosol and permeabilized bovine glomerulosa cells, establishing Ins-1,3,4,6-P_4 as a link between agonist-stimulated $InsP_3$ metabolism and the higher inositol phosphates ($InsP_5$ and $InsP_6$) recently detected in the adrenal gland and other mammalian tissues. In addition to the conversion of Ins-1,3,4,6-P_4 to $InsP_5$, two other $InsP_4$ isomers were detected and characterized in AII-stimulated adrenal cells. These were identified as Ins-3,4,5,6-P_4, shown to be an additional and major precursor of $InsP_5$, and its stereoisomer, Ins-1,4,5,6-P_4, which were found to be formed by dephosphorylation of $InsP_5$. AII stimulation was also found to cause a short-term increase in conversion of $InsP_5$ to Ins-1,4,5,6-P_4, the first evidence for agonist-regulated control of $InsP_5$ breakdown. In addition, AII caused a progressive elevation of Ins-3,4,5,6-P_4 and $InsP_5$, thus providing further evidence for a connection between agonist-induced phosphoinositide hydrolysis and the production of higher inositol polyphosphates in the adrenal glomerulosa cell. These findings have provided compelling evidence for the existence of a major contribution from the metabolites of Ins-1,4,5-P_3, the primary signaling molecule responsible for calcium mobilization, with the slowly formed higher inositol polyphosphates, compounds for which alternative biosynthetic pathways may also exist via intermediates of as yet undetermined origin.

Previous investigation of the molecular basis of hormone action during granulosa cell differentiation included evaluation of the functions and mechanisms of action of pituitary hormones and growth factors. In such studies, TGF-beta was found to exert bifunctional actions on the maturation of granulosa cells, and to modulate FSH-induced stimulation of cAMP formation, steroidogenesis, and LH receptor expression. TGF-beta also accelerated the maturation of both follicle-enclosed oocytes and cumulus-oocyte complexes, with significant increases in the rate of germinal vesicle breakdown. Other growth factors including IGF-I, IGF-II, and EGF also stimulated germinal vesicle breakdown. For further studies on growth control of rat granulosa cells, which do not undergo division in culture, a continuous cell line was derived by transformation of granulosa cells with an SV40 tsA255 mutant, which has a temperature-sensitive mutation in the gene required for maintenance of transformation. This granulosa cell-derived line (RGA-41S) exhibits temperature-regulated proliferation behavior with unrestrained growth and a transformed phenotype at the permissive temperature (33 °C) and a differentiated non-transformed phenotype with growth arrest at the nonpermissive temperature (40 °C). The cultured RGA-41S cells were found to produce IGF-I mRNA and to secrete IGF-I, and also to express receptors for IGF-I as well as the corresponding mRNA. These cells provide a model system for studies on the paracrine and autocrine actions of IGF-I in the growth and differentiation of ovarian and other epithelial cells.

In normal granulosa cells, studies were commenced on the regulation of the ovarian renin-angiotensin system, employing solution hybridization procedures to measure the steady state levels of renin and angiotensinogen mRNA during maturation and

stimulation of granulosa cells by gonadotropins and growth factors in vitro. Also, based on recent findings that growth hormone releasing hormone (GHRH) increases the incidence of ovulation in women undergoing gonadotropin therapy for infertility, and that immunoreactive GHRH is present in the gonads, local actions of GHRH on granulosa cell function were evaluated in vitro. In initial studies, GHRH exerted consistently stimulatory effects on cyclic AMP production and steroidogenesis in the granulosa cell, revealing a local regulatory mechanism with the potential to synergize with FSH at the ovarian level. These studies are being extended to include characterization of the GHRH receptor of the ovary and its relationship to the receptors known to mediate the actions of VIP and related peptides upon ovarian function.

The Section on Endocrine Physiology (Dr. Greti Aguilera) investigates physiological and pathological aspects of circulatory homeostasis and neuroendocrine regulation, including mechanisms of adaptation to stress. This program also includes studies on the role of the renin-angiotensin system in the regulation of mineralocorticoid secretion and blood pressure, and the effects of AII in other systems including the pituitary and gonads. A major purpose of this project is to analyze the physiological functions of the renin-angiotensin system, including the effects of AII in circulatory homeostasis, fetal development, and control of pituitary and gonadal function.

AII mediates the increase in aldosterone secretion during sodium restriction, but the adrenal effects of the peptide are dependent on the sensitivity of the glomerulosa zone to AII. Previous studies in the rat have demonstrated that the adrenal responsiveness to AII depends on trophic effects of the peptide and the modulatory effect of other regulators such as dopamine, atrial natriuretic factor (ANF) and somatostatin (SRIF). Elucidation of the mechanisms by which these factors modulate the effects of AII requires a precise knowledge of the mechanism of action of AII. The cellular response to AII in the adrenal glomerulosa zone includes calcium mobilization and phospholipid turnover, with possible activation of protein kinase C (PKC). However, the relative importance of these events in stimulation of steroidogenesis is not understood. This is particularly unclear in regard to PKC since phorbol esters, known stimulators of the enzyme, are ineffective in stimulating aldosterone production under most experimental conditions.

The role of PKC in the steroidogenic action of AII was investigated by depletion of endogenous PKC during prolonged incubation of glomerulosa cells with phorbol ester. Direct measurement of PKC in rat glomerulosa cell cytosolic and detergent-solubilized membrane fractions, purified by DEAE cellulose chromatography, showed that basal PKC activity was 2 to 3-fold higher in cytosol than in membranes. After incubation of the cells with AII for up to 60 min, PKC activity in the cytosol progressively decreased by up to 27%, while the membrane showed a transient increase of 15% at 15 min, returning to basal by 60 min. Incubation of cells with 100 nM 12-0-tetradecanoyl-phorbol-13-acetate (TPA) resulted in transient translocation of PKC activity to the membrane (15 min) which was followed by a 70 to 90 decrease in total cellular enzyme activity after 2 hr. In PKC-depleted cells, the aldosterone response to ACTH was increased by 25% but AII-stimulated steroidogenesis was unchanged. In contrast, in cells in which PKC was translocated to the membrane by 15 min preincubation with TPA, the aldosterone response to AII was enhanced by 40% while the response to ACTH was reduced by 30%; under these conditions membrane PKC levels rapidly returned to basal. However, the changes in aldosterone response were still evident when addition of AII or ACTH was delayed for up to 30 min after removal of TPA, indicating a persistent modification in

the cell membrane secondary to PKC activation. Aldosterone responses to potassium were not altered by preincubation of the cells with TPA. The inactive phorbol ester analog, 4α-hydroxyphorbol-12,13-dibutyrate, had no effect on steroid responses to either stimulus. The small but significant translocation of PKC activity from cytosol to membrane following treatment of rat adrenal glomerulosa cells with AII suggests that AII activates PKC. However, the fact that aldosterone responses to AII are potentiated during TPA-induced PKC translocation to the membrane indicates that AII and phorbol esters do not share the same mechanism of action in the regulation of steroidogenesis. In addition, the full aldosterone response to AII despite marked cellular PKC depletion followed prolonged preincubation with TPA argues against the involvement of PKC in the stimulation of aldosterone production by AII. It is possible that activation of PKC by AII mediates effects other than steroidogenesis, such as trophic maintenance and/or growth of the adrenal glomerulosa cell.

The distribution and role of AII receptors during early and late embryonic development was further studied in whole mouse blastocysts and membrane-rich fractions from rat conceptuses. In early mouse embryos there was no detectable AII binding, but late gestation rat fetuses shows specific and high affinity binding, and the concentration of AII sites was similar to membranes from whole and eviscerated fetuses. Scatchard analysis of the binding data indicated Kd values ranging between 0.7 and 0.9 nM. Binding was first detectable at day 10 of fetal life and increased progressively with gestational age. AII receptors in skin and skeletal muscle membrane showed an 80% decrease in AII receptors one day after birth and subsequently became almost undetectable in the adult. The functional significance of these binding sites would depend on the availability of the agonist ligand, angiotensin II. Immunoreactive AII-like material with chromatographic properties identical to an AII standard was detected in 10-day fetuses and shown to be bioactive in an AII receptor assay, providing evidence for potential activation of the fetal receptors by the cognate ligand, AII.

Localization of renin mRNA in fetal tissues was analyzed by *in situ* hybridization using an antisense ^{35}S-labeled riboprobe transcribed from a mouse renin 2 cDNA clone. Hybridization to tissue sections showed high intensity staining in the fetal kidney and adrenal cortex. Northern blot analysis confirmed the expression of high levels of renin mRNA in the fetal kidney. However, only low and inconsistent labeling was observed in the epidermis. The physiological effects of AII in the fetus were investigated in cultured fibroblast-like cells from fetal skin, which contain abundant AII receptors with properties identical to those described in fetal membranes. Stimulation of the cultured cells with AII resulted in increases in cytosolic calcium and inositol phosphate formation, indicating that the binding sites correspond to receptors linked to intracellular responses. The abundance, timing of expression, and unique localization of functional AII receptors in the fetus suggest a role for AII in fetal development.

Studies on the hypothalamic-pituitary system have focused on the properties and regulation of corticotropin releasing factor (CRF) receptors and the mechanisms of interaction between CRF and other regulators of ACTH secretion, with emphasis on adaptation to stress. The biphasic ACTH responses to prolonged immobilization were previously shown to be accompanied by decreases in pituitary CRF receptors and hypersensitivity of the pituitary to CRF infusion and a second novel stress. Extension of these studies using a more mild form of stress, intermittent immobilization for 2.5 hr daily, showed complete desensitization of the ACTH response after the first 4 days of immobilization, while plasma glucocorticoids were markedly increased. Despite a decrease in pituitary CRF receptors, ACTH responses to a novel stress, but not to CRF

infusion, were enhanced. These responses were similar to those observed in types of human depression in which hypercortisolemia is a characteristic feature. CRF receptors in the intermediate pituitary were unchanged during immobilization stress. In contrast to the changes during immobilization stress, CRF receptors were unaltered in the anterior pituitary during cold stress, but were markedly increased in the intermediate lobe. The binding characteristics of CRF receptors determined in membranes from the neurointermediate lobes were almost identical to those in the anterior pituitary. Analysis of the solubilized cross-linked CRF-receptor complex in different target tissues by gel electrophoresis showed similar properties in both pituitary lobes, with a molecular weight of about 70 kDa. It is known that CRF receptor occupancy results in activation of adenylate cyclase. In addition, experiments showing that pharmacological inhibition of protein kinase C inhibits ACTH release suggest that the CRF receptor may be also linked to a calcium/phospholipid-dependent transduction system.

The Section on Molecular Endocrinology (Dr. Maria Dufau) investigates the molecular basis of peptide hormone action, with particular emphasis on the characterization of gonadotropin receptors, activation of steroid biosynthesis in gonads and adrenal, and analysis of the biological activity of circulating gonadotropins. A major aspect of this program is concerned with the characterization of gonadal gonadotropin and prolactin receptors, and of the physical and functional relationships of the LH receptor site and adenylate cyclase.

The Nb2 lymphoma cell is dependent upon lactogens for proliferation and provides an invaluable model for the study of prolactin (PRL) receptor coupling functions and messengers for prolactin's multiple biological actions. Recent studies in the Section demonstrated that the proliferative effect of prolactin was modulated by pertussis and cholera toxins, and that these changes were independent of the effects of cyclic AMP on prolactin-induced mitogenesis, indicating that G-proteins could be involved in signal transduction steps. It was also found that exposure of Nb2 cells to prolactin caused time- and dose-dependent changes in the ability of specific 38 kDa and 41.5 kDa membrane proteins to be subsequently ADP-ribosylated by pertussis and cholera toxins, respectively. These include rapid reduction of the 41.5 kDa substrate for cholera toxin and a marked increase of the 38 kDa substrate for pertussis toxin. The early changes observed in cholera toxin-induced ADP-ribosylation suggest a rapid and direct effect at the membrane level, presumably involving the interaction of prolactin with its receptor, dissociation of the α-subunit from the $\beta\gamma$-complex of the trimeric G-protein and activation of the α-subunit. This may account for the reduced susceptibility to ADP-ribosylation after hormone treatment, as suggested in certain other systems. The changes revealed by pertussis toxin may result from structural changes of the putative G protein from interaction with the activated lactogen receptor, and/or may involve preferential increases in toxin-sensitive G-protein synthesis. This study has provided the first demonstration of a direct effect of prolactin on G-proteins, and has suggested that such regulatory proteins are involved in the signal transduction mechanism by which occupancy of the prolactin receptor leads to proliferation of Nb2 lymphoma cells. The influence of prolactin on the G-proteins of Nb_2 lymphoma cells has opened an avenue for elucidation of lactogen receptor-coupled functions in mitogenesis, an approach that could have general application for signalling studies in other cells expressing the diverse actions of prolactin in its several target tissues.

The purified ovarian LH/hCG receptor was identified as a monomeric protein (Mr 80,000) and its homogeneity was confirmed by microsequencing. The N-terminal peptide sequence was NH2-R-E-L-S-G-S-R-X-REP-D-X-D-X-A-P-D-G. A sequence derived

from cyanogen bromide cleavage (P-L-V-G-I-S-N-Y) was used to raise antibodies and to screen genomic and cDNA libraries. A cDNA clone was isolated with 61% overall nucleotide similarity with the recently cloned LH receptor, and the possibility that this molecule could be a yet unidentified receptor or channel is under investigation. The purified testicular and ovarian LH/hCG receptors were shown to be phosphorylated *in vitro* by the catalytic subunit of cAMP dependent protein kinase. The influence of hormone in the phosphorylation event was related to stages of trophic hormone binding ("early loose binding and "late" tight binding) which through conformational changes affected the rate of phosphorylation. Since the LH/hCG receptor of testis and ovary is only available in microgram quantities, the use of phosphorylated receptors was employed to facilitate structural studies. In initial studies using reduced phosphorylated receptor, we demonstrated that the LH/hCG receptor contains sialylated N-linked carbohydrate chains of the biantennary type, the hybrid type or both, and that glycosylation could account for the size difference of ovarian and testicular receptors. Conditions were established for complete enzyme digestion (neuraminidase, N-Glycanase) of phosphorylated receptors, with conditions for preservation of hormone binding activity in the native blotted receptor. Studies on blotted receptors revealed components with Mrs from 90-92 kDa to 82 kDa (testis) and 80-85 kDa (ovary), with a decrease to 60 kDa in both tissues after N-glycanase treatment. Subsequently, studies on the binding of ^{125}I-hCG to blots of desialylated or deglysosylated native receptor after SDS/PAGE demonstrated that the terminal sialic acid of the glycosidic chain was not involved in hormone binding. However, the removal of glycosyl residues by N-Glycanase caused a major decrease in hormone binding, indicating the participation of N-linked carbohydrates in agonist-receptor interactions.

Luteinizing hormone is the major regulator of Leydig cell differentiation and steroidogenic function. A number of hormones produced by the Leydig cell (e.g. estrogen, angiotensin, CRF, vasopressin) and the tubular compartment (inhibin, TGF beta), can influence both acute and chronic actions of LH. Conversely, hormones produced in the Leydig cells modulate tubular function (e.g. androgen, β-endorphin, and oxytocin). The LH stimulatory actions of LH on Leydig cell function can be negatively influenced by the effects of angiotensin II that are exerted through the guanyl nucleotide inhibitory unit of adenylate cyclase. Recent studies in the Section have revealed a novel inhibitory action of corticotrophin releasing hormone (CRF) through specific high-affinity low-capacity receptors in the Leydig cells. This process involves a pertussis toxin insensitive guanyl nucleotide regulatory unit with interaction between signalling pathways and resulting inhibition of LH-induced cAMP generation and consequently of steroidogenesis. In contrast to its actions in brain, pituitary and other peripheral tissues, CRF receptors in the Leydig cell are not coupled to Gs and stimulation of adenylate cyclase. Rather, the inhibitory action of CRF in the Leydig cells is exerted through direct or indirect activation of protein kinase C, at the level of one or more components of the adenylate cyclase system. These studies have demonstrated a novel mechanism of action for CRF, probably mediated by a CRF receptor subtype, and have highlighted the importance of interactions between different signal transduction pathways in cellular homeostasis. Also, these observations have demonstrated that CRF has potent antireproductive effect at the testicular level, and since CRF is synthesized in the testis and is present in Leydig cells, it is likely that locally produced CRF could exert negative autocrine modulation on the stimulatory action of luteinizing hormone on Leydig cell function.

Physiological increases in endogenous LH action cause positive regulation of membrane receptors and steroidogenesis while major elevations in circulating gonadotropin can

induce down-regulation of LH receptors and desensitization of steroid responses in the adult cell. Gonadotropin-induced desensitization in adult rat testes include an estrogen-mediated steroidogenic lesion of the microsomal enzyme(s) 17α-hydroxylase/17-20 desmolase. For further understanding of the structure and regulation of this key enzyme of the androgen pathway, the rat P450-17α cDNA was cloned and sequenced. This cDNA expressed 17α hydroxylase/17,20 desmolase activities when transfected into COS cells. From the deduced amino acid sequence, two transmembrane regions were identified. The first of these was a signal peptide for insertion in the ER, and the second, which was separated from the first transmembrane region by 122 amino acids, could serve as the stop-transfer sequence. The structure of the carboxyterminal non-transmembrane region is composed of 4 hydrophobic clefts, of which cleft II would contain the putative steroid binding site for both hydroxylase and lyase activities. Gonadotropin stimulation and desensitization of P450-17α dependent enzymes (17α-hydroxylase/17,20 desmolase) are related to the levels of P450-17α mRNA, which show concomitant increases and decreases during the stimulatory and desensitization phases of LH action in the Leydig cell.

LH is released from the pituitary gland into the circulation as episodic pulses of high biological activity. This episodic mode of secretion may serve to obviate gonadotropin-induced desensitization of hormone-responsive gonadal cells. The exact nature of glandular secretory events is difficult to discern *in vivo*, since underlying patterns of hormone release are confounded by the effects of metabolic clearance. Using a deconvolution model that allows the calculation of endogenous clearance kinetics and secretory rates simultaneously, multiple parameter deconvolution disclosed an endogenous bioactive LH half-life of 53 ± 5.4 min and an endogenous production rate of 0.48 ± 0.06 mIU/ml/min. The bioactive LH secretory burst with half-duration of 12.2 ± 1.5 min occurred at intervals of 56 ± 1.3 min and achieved amplitudes of 2.1 ± 0.26 mIU min. LH secretory bursts were positively correlated with the duration of the subsequent interpulse interval. Application of the deconvolution algorithm in conjunction with the rat Leydig cell testosterone bioassay revealed the presence of distinct burst-like secretory events acted on by exponential metabolic clearance mechanisms, and provided a good model for analysis of bioactive plasma hormone concentration profiles *in vivo*. Such multifaceted changes might not be readily apparent using deconvolution techniques requiring known half-lives/or conventional pulse analysis of plasma hormone concentration. The present analysis indicates that endogenous clearance mechanisms provide a mechanism to extend the defined short-lived pituitary secretory events. The multiple parameter deconvolution approach may help to unmask pathophysiological states with alterations in endogenous hormone as well as clearance *in vivo*.

In related studies, the time-dependent effects of estrogen on the kinetics of GnRH's self-priming action on the pituitary gland were analyzed in post-menopausal women on intravaginal estradiol replacement. The self-priming action of GnRH on bioactive LH release is maximal after 5 to 10 days of estrogen exposure and can be observed whether it is defined as increase in the percentage amplitude of a second peak of bioactive LH provoked by exogenous GnRH (10 micrograms,i.v) compared to the first LH peak (given at 2-hourly intervals) or as an incremental increase. These findings indicated that estrogen regulates the human pituitary gland's responsiveness to GnRH, such that enhanced secretion of biologically active gonadotropin occurs during serial exposure to GnRH stimulation. The finding of GnRH self-priming induced by estradiol is of importance to an understanding of possible mechanisms governing the generation of the preovulatory gonadotropin surge.

The Section on Adrenal Cell Biology (Dr. C. Strott) investigates the physiology and regulation of adrenal steroidogenesis, by characterization of cellular steroid binding proteins and soluble factors which mediate steroidogenic responses to ACTH, and analysis of cellular mechanisms of cholesterol utilization in steroid biosynthesis. The Section is also interested in the development of adrenocortical zonation and the regulation of adrenal steroidogenesis, and is currently concentrating on two areas of research: 1) adrenocortical calmodulin, calcium- and calmodulin-binding proteins, protein kinase systems, and the post-translational modification of proteins; 2) purification, immunology, and functional activity of soluble and membranous adrenocortical proteins including steroid-binding proteins.

The molecular mechanisms involved in the action of ACTH (a key stress hormone) on the adrenal cortex are poorly understood. What is known is that ACTH binds to a cell surface receptor and stimulates adenylate cyclase leading to an increase in intracellular cAMP and the activation of cAMP-dependent protein kinase. The steroidogenic response to ACTH can be separated into acute and subacute aspects. The acute response(sec-min) occurs primarily at the level of mitochondria and involves the translocation of cholesterol to the inner mitochondrial membrane followed by the conversion of cholesterol to pregnenolone (rate-limiting). The subacute response (hours) appears to occur at the level of the genome and involves synthesis of enzymes and co-factors. Both the acute and subacute responses to ACTH are mediated by cAMP and cAMP-dependent protein kinase, and are dependent on the synthesis of protein. The existence of adrenocortical steroidogenic regulatory proteins has been proposed but none has been identified as yet. The role of Ca^{2+} in ACTH action is complex; Ca^{2+}-regulated protein kinases may be involved. Thus, the molecular mechanisms involved in ACTH action remain to be elucidated. Pregnenolone, the product of the rate-limiting step in steroidogenesis, is poorly soluble in aqueous media and must be translocated between intracellular compartments; the translocation process is not understood. Furthermore, a biological role for pregnenolone other than that of a precursor has not been established. Progesterone, which is derived from pregnenolone and closely related, not only serves as a key steroid intermediate itself but is a ligand for a member of the steroid receptor super gene family. To examine the complex process of ACTH action, the possible involvement of specific steroid-binding proteins has been explored. A 34 kDa cytosolic pregnenolone-binding protein (P_5BP) has been identified, purified and characterized. The P_5BP has multiple forms with PI's of 6.5, 5.5, 5.4 and 5.2. Individual isoforms have been isolated, antisera generated and immunological identity demonstrated. Pregnenolone-binding activity is abolished by treatment with alkaline phosphatase. The P_5BP isoform patterns in the outer ACTH-responsive and inner ACTH-unresponsive zones are distinct and markedly altered by phosphatase treatment. The N-terminus of P_5BP is blocked, necessitating determination of internal peptide sequences after partial tryptic digestion.

These studies have shown that pregnenolone, the product of the rate-limiting step in steroidogenesis, binds specifically to a non-catalytic cytoplasmic protein (P_5BP); furthermore, the binding-activity was found to be regulated by phosphorylation/ dephosphorylation. The physiological role of P_5BP, although undoubtedly important, remains to be clarified. There are several possibilities: 1) the P_5BP could function as a transporter for pregnenolone which is poorly soluble in an aqueous medium; 2) the P_5BP could act to regulate cholesterol side-chain cleavage (e.g. the dephosphorylated, pregnenolone-unbound form could suppress activity while the phosphorylated, pregnenolone-bound form relieves the inhibition or *vice versa*); 3) the P_5BP may be necessary for further metabolism of pregnenolone; 4) since pregnenolone can be

metabolized by several routes (at least 6), perhaps the P_5BP serves to ensure that pregnenolone is metabolized by a specific route; 5) pregnenolone may have a biological function in its own right that is carried out when bound to a specific protein.

Progesterone, which is derived directly from pregnenolone, has been found to bind specifically to an acidic nuclear protein (P_4BP) of MW 75-79 kDa; the binding activity appears not to be regulated by phosphorylation/dephosphorylation. The nuclear location of P_4BP places the protein in the steroid receptor category, although the biochemical behavior of P_4BP is different from that of the classical progesterone receptor described in uterine and breast tissue, as well as the hen oviduct. As with P_5BP, the physiological role of the P_4BP remains to be elucidated. Perhaps the P_5BP, which appears to be primarily, if not solely, in the cytoplasmic compartment, is involved in the acute response to ACTH. In contrast, the P_4BP, which appears to be located in the nucleus, is involved in the subacute response to ACTH. It is interesting to speculate as to whether both binding proteins are members of the steroid receptor super gene family.

The Section on Molecular Structure and Protein Chemistry (Dr. H.-C. Chen) conducts research on the analysis, synthesis, and structure-function relationships of biologically active peptides and proteins. This includes the identification and synthesis of unusual structures and sequences in amino acids and peptides, and the development of new techniques for peptide sequencing and synthesis. Of particular interest are the structural design, chemical synthesis, and modification of molecules important to reproductive and developmental biology. A major component of this project focuses on the role of carbohydrate structures of human chorionic gonadotropin (hCG) in the subunit association kinetics and effect on *in vivo* biological activity. Re-evaluation of previous studies on the reassociation kinetics of hCG subunits, measuring the fluorescence enhancement of 1-anilino-naphthalene-8-sulfonate that occurs with hCG subunit association, has revealed that the reassociation rate for deglycosylated subunits is 23-fold faster than that of the native hCG molecule. An initial time lag was noted during the reassociation. The fit of the data against four different models was tested and was found to be most consistent with a model which predicts that the order of the reaction and the extent of lag depend on the concentration of the deglycosylated α-subunit. The rate limiting step appears to be the conformational transition of the deglycosylated α-subunit. Although a conformational transition of the β-subunit may also occur, the significance of this process as rate determining is minimal in all models tested. These findings have led to the proposal that hCG may be assembled as a non-deglycosylated form in living cells, and that the excess secretion of the carbohydrate-rich non-associable free hCG-α-subunit in disease states may be caused by over-glycosylation of the α-subunit.

In related studies, HF-deglycosylated hCG has been purified and employed for studies of the pharmacokinetics and stimulation of testosterone production in the Cynomolgus monkey *in vivo*. These studies have shown that the removal of carbohydrate residues from hCG causes profound changes in the rate constant of hormone clearance with no change in the initial volume of distribution. The deglycosylated hCG also remains a full agonist at the LH receptor in the primate *in vivo*, consistent with previous findings *in vitro* systems.

During the current year, structural design, chemical synthesis, conformational studies, and the mode of action of the vertebrate peptide antibiotics, magainins, have been studied. A major objective of this research is to elucidate the manner in which alpha-

helicity, amphiphilicity, and stability to proteases contribute to broad spectrum antimicrobial activity without causing toxicity to mammalian tissues. Substitution of low propensity residues (Gly^{13}, Gly^{18} and Ser^{8}) in the natural magainin 2 sequence by Ala enhanced α-helical structure and gave analogues with increases of up to two orders of magnitude in antimicrobial activity, and no appreciable increase in hemolytic activity over the natural magainins. Although more than 500 analogues have been synthesized by various laboratories, none surpasses the level of antimicrobial activity of our Ala-substituted analogues. Studies with eight magainin 2 amide analogues of D-Ala and L-Ala permuted at positions 9, 13, 18 have revealed that D-Ala modification reduces α-helical potential. In evaluating antimicrobial activity, modification at position 13 or 18 is less important than at 9, and the cumulative effect of a D-Ala modification at 9 and 13 is more deleterious than at 13 and 18. These results suggest that α-helix in the N-terminal region is most important in eliciting biological activity, which is probably due to the presence of positive charges which can interact with membrane phospholipids.

The ability of magainins to inhibit the growth of a wide variety of bacteria and to cause lysis of protozoa has prompted the suggestion that the peptides act upon lipid membranes. It had been proposed that magainins induce anion selective channels in black lipid membranes. However, current studies have shown that in cytochrome oxidase lysosomes, membrane potential is dissipated and respiratory control released; the latter requires proton translocation. In comparing the magainin 2 amide and a potent analogue, magainin A, both peptides were found to decrease the membrane potential of E. coli cells. However, magainin A caused persistent drops of membrane potential whereas magainin 2 amide was active only at much higher concentrations. Magainin A was also 40 times more active than magainin 2 amide in inhibiting the growth of E. coli. In cytochrome oxidase liposomes, magainin A was approximately 20% more effective than magainin 2 amide at a peptide concentration causing half-maximal stimulation of respiration. These results suggest that the difference in antimicrobial activity of these two peptides is not due simply to a substantial difference in their ability to interact with lipid membranes. To examine the cause of this difference, the relative susceptibility of magainin analogues to pronase inactivation in uncoupling the respiration rate of cytochrome oxidase was examined. Pronase was able to reverse the uncoupling caused by magainin 2 amide and to restore the coupled rate, but did not reverse the uncoupling activity of magainin A. This observation suggests that magainin A, which is a strong former of α-helical structure, interacts with lipids much more readily than magainin 2 amide, and is thereby less susceptible to proteolysis. Such resistance to proteolysis may be in part responsible for the differences in antimicrobial activity of magainins, and for the increased biological activity of the potent analogues.

The Section on Metabolic Regulation (Dr. K.-P. Huang) studies the role of protein kinases and phosphorylation-dephosphorylation of proteins in the regulation of cellular functions. Also, the regulation and hormonal control of glycogen metabolism, and the activities of glycogen synthase and phosphorylase kinase. The receptor-mediated turnover of membrane phospholipids plays an important role in the regulation of many cellular functions. Inositol 1,4,5-trisphosphate triggers the release of calcium from an intracellular nonmitochondrial pool, whereas diacylglycerol activates protein kinase C to modulate numerous cellular responses. This signal-transduction pathway has been implicated in the regulation of cell growth, differentiation, gene expression, hormone and neurotransmitter release, cell-surface receptor function, and cellular metabolism.

Phosphorylation-dephosphorylation of proteins is one of the most important mechanisms for the regulation of cellular functions. Protein kinase C (PKC), a Ca2+/phospholipid-

dependent protein kinase, has emerged as a pivotal regulatory element for cell growth, differentiation, gene expression, hormone secretion, cell surface receptor function, and cellular metabolism. This protein kinase can be activated by diacylglycerol, a second messenger generated by signal-induced breakdown of phosphoinositides. In addition, it has been identified as a receptor for tumor-promoting phorbol esters which elicit pleiotropic responses comparable to those stimulated by many hormones and growth factors. Three major isozymic forms of PKC have been identified from mammalian brains. Polyclonal and monoclonal antibodies against these enzymes were prepared and utilized to determine their immunochemical characteristics and tissue distribution. These enzymes were found to have distinct tissue, cellular, and subcellular localizations, and to be differentially expressed during development.

Although protein kinase C isozymes have been implicated in the regulation of diverse cellular function; the role of each of these enzymes is unknown. One of the approaches to investigate this problem is to identify a specific inhibitor or activator for each enzyme. Since protein kinase C is dependent on Ca^{2+} and phospholipid for activity, the actions of these effectors on the kinase were investigated by a "preincubation" protocol to determine the resulting changes in kinase activity and phorbol ester binding. It was found that acidic phospholipids such as phosphatidylserine (PS), phosphatidic acid, phosphatidylglycerol, and cardiolipin could differentially inactivate PKC I, II, and III in the absence of divalent cations. The phospholipid-induced inactivation of PKC was concentration- and time-dependent and only affected the kinase activity without influencing the phorbol ester binding. PKC I was most susceptible to the phospholipid-induced inactivation and PKC III was the least. Addition of divalent cations such as Ca^{2+} or Mg^{2+} suppressed the phospholipid-induced inactivation of PKC. In the absence of divalent cation, PKC I, II, and III all formed complexes with PS vesicles, although to a slightly different degree, as analyzed by molecule sieve chromatography. [^{3}H]PDBu binding to PKC I, II, and III was recovered after chromatography; however, the kinase activities of all three enzymes were greatly reduced. In the presence of Ca^{2+}, all three PKCs formed complexes with PS vesicles and both the kinase and phorbol ester-binding activities of PKC II and III were recovered following chromatography. Under the same conditions, the phorbol ester binding activity of PKC I was also recovered, but the kinase activity was not. The phospholipid-induced inactivation of PKC apparently results from a direct interaction of phospholipid with the catalytic domain of PKC; this interaction can be suppressed by divalent cations. In the presence of divalent cations, PS interacts preferentially with the regulatory domain of PKC, resulting in activation of the kinase.

The structure/function relationships of PKC were analyzed by deletion analysis. PKC contains a regulatory domain at the NH_2-terminal half and a catalytic domain at the carboxyl-terminal half of the molecule. Within the regulatory domain there are two cysteine-rich Zn^{2+}-finger structures and a pseudosubstrate region. The regulatory domain was previously shown to contain phorbol ester and phospholipid binding activity. To define further the structure/function relationships, deletion analysis of the cDNAs was performed to determine the region of the PKC molecule important for conferring the regulatory function. The modified cDNAs were expressed in COS cells and the products were purified by column chromatography and analyzed by immunochemical and biochemical methods. Deletion of either cysteine-rich region has no effect on the binding of phorbol ester, whereas deletion of both cysteine-rich Zn^{2+}-finger regions eliminates the phorbol ester binding completely, indicating that the Zn^{2+}-finger structure is important for phorbol ester binding. Deletion of the phorbol ester-binding domain or the pseudosubstrate region, an area that presumably binds to the active site

of PKC to keep the enzyme in an inactive form in the absence of activator, results in loss of the kinase activity. It was noted that the expression products of the modified cDNAs always associated with the particulate fraction, indicating that an unusual modification of the enzyme takes place during biosynthesis. It is possible that a unique "clearance" mechanism is functioning to convert the defective enzyme into a separate pool to prevent interference with the function of the intact kinase.

PKC inhibitors have been found to be very useful in defining the functional role of this enzyme in cellular regulation. Recently, it was demonstrated that a PKC inhibitor (H-7) can prevent HIV infection of cultured T-cells by inhibition of CD4 phosphorylation, a process believed to be essential for viral entry. Suramin, an anti-HIV reverse transcriptase agent, was found to exert biphasic effects on PKC; it activates these kinases at low concentrations (< 40 μM) but inhibits them at higher concentrations. This effect of suramin also exhibits selectivity among the different PKC isozymes. PKC I is most sensitive to activation by low concentrations of suramin and is most sensitive to inhibition by high concentrations of this agent. Among all the PKC inhibitors identified so far, suramin appears to be the first to display selectivity against the different isozymes. Since many trans-membrane signaling events act through PKC, it is likely that some of the functional specificity of cellular responses is transmitted by different PKC isozymes. This may also be true for the HIV infection process. Thus, the development of PKC isozyme-specific inhibitors should be useful as tools in the study of PKC-mediated processes and as potential therapeutic agents.

Previous studies show that PKC isozymes (types I, II, and III) have distinctive neuronal localizations in the cerebellum. The expression of these isozymes during the postnatal development of the cerebellum was followed by immunochemical analysis. By immunoblot analysis, type I PKC was found to be low within two weeks after birth, showed an abrupt increase between two to three weeks, and leveled off afterward. By immunofluorescent staining, the type I PKC-specific antibody recognized the cell bodies and dendrites of Purkinje cells. The increase of this isozyme between two to three weeks of age correlates with the spreading of Purkinje cell arborization, at which time the bulk of synaptogenesis between dendritic spines and axons of granule cells occurs. Both type II and III PKC's were present in granule cells. At birth, the level of type II PKC was relatively high compared to that of type III PKC, and the type II PKC-specific antibody stained the granule cell precursors in the external layer more heavily than did the type III PKC-specific antibody. The level of type II PKC declined slightly after birth and increased again at one week and plateaued after three weeks, whereas that of type III PKC increased gradually until leveling off after three weeks. Throughout development the type III PKC-specific antibody also stained the cell bodies of Purkinje cells but not their dendrites. These results demonstrate that the developmental expression of PKC isozymes is under separate control, and their distinct cellular and subcellular localizations suggest their unique functions in the cerebellum.

As described above, both type II and III, but not type I, protein kinase C (PKC) isozymes are present in the granule cells of adult rat cerebellum. Type II and III PKCs, but not type I, were also present in the primary cultures of granule cells prepared from 8-day old rats. However, the expression patterns of these isozymes were different. Except for a slightly elevated level at 4-5 days, type II PKC was expressed at a relatively stable level throughout 13 days in culture. On the contrary, type III PKC was expressed at a low level initially and the content increased as the culture continued up to 13 days. Treatment of the 8-day cultures, when type II PKC was the predominant species, with PMA resulted in the down-regulation of PKC,

whereas PKC in the 13-day culture, when type III PKC was predominant, was less susceptible to down-regulation by PMA. Treatment of the cultures with glutamate, the excitatory amino acid, caused a reduction of both type II and III PKCs in the cytosol and an increase of these isozymes in the membrane fraction. Although the time course and extent of translocation varied from culture to culture, accumulation of type II, but not type III, PKC in an unique membranous fraction which was not extractable by 0.5% NP40 containing buffer, was consistently observed. These results suggested that type II and III PKCs, with their dissimilar expression patterns and responses to glutamate, may also be endowed with dissimilar and specific functions in granule cells of cerebellum.

Phosphorylation of myosin light chain is thought to play an important role in the regulation of smooth muscle contraction. Phosphorylation of the light chain by myosin light chain kinase at Ser-19 and Thr-18 results in an increase in the actin-activated Mg-ATPase activity. In contrast, phosphorylation of the light chain at Ser-1, Ser-2, and Thr-9 by PKC counteracts the increase in the ATPase activity caused by myosin light chain kinase. In order to test the effect of proteolysis as a mechanism for the activation of PKC, the phosphorylation of myosin light chain by PKC was compared with that by the protease-degraded spontaneously active enzyme, PKM. This study revealed that PKM phosphorylated the light chain to a higher stoichiometry than PKC. Analysis of the ^{32}P-labeled tryptic peptides derived from the light chain phosphorylated by PKM revealed that both the sites phosphorylated by PKC and myosin light chain kinase were phosphorylated by PKM alone. The PKM-mediated phosphorylation of both sets of phosphorylation sites was not affected by an inhibitor, ML-9, nor the activators of myosin light chain kinase, Ca^{2+} and calmodulin. These results provide evidence that the substrate specificity of PKC could be altered following its conversion to PKM.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00022-16 ERRB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Renin-Angiotensin System and Aldosterone Regulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	G. Aguilera	Head, SEP	ERRB, NICHD
Others:	K.J. Catt	Head, SHR	ERRB, NICHD
	M.A. Millan	Sr. Staff Fellow	ERRB, NICHD
	S. Rocco	Visiting Fellow	ERRB, NICHD
	S. Zemel	Guest Researcher	ERRB, NICHD

COOPERATING UNITS *(if any)*

Contract for preparation of adrenal and pituitary cells N01-HD-0-2806

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Endocrine Physiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to analyze physiological and pathological aspects of the renin-angiotensin system, including the effects of AII in circulatory homeostasis, fetal development pituitary and gonadal function. AII mediates the increase in aldosterone secretion during sodium restriction, but the adrenal effects of the peptide are dependent on the sensitivity of the glomerulosa zone to AII. Previous studies in the rat have demonstrated that the adrenal responsiveness to AII depends on trophic effects of the peptide and the modulatory effect of other regulators such as dopamine, atrial natriuretic factor (ANF) and somatostatin (SRIF). Elucidation of the mechanisms by which these factors modulate the effects of AII requires a precise knowledge of the mechanism of action of AII. The cellular effects of AII include calcium mobilization and phospholipid turnover. In isolated rat adrenal glomerulosa cells, AII was shown to cause translocation and presumably activation of protein kinase C (PKC). Studies directed to determine the role of PKC in the steroidogenic action of AII provided two lines of evidence indicating that PKC does not mediate the stimulation of aldosterone secretion by AII. First, depletion of endogenous PKC by prolonged incubation with phorbol esters had no effect on AII-stimulated aldosterone responses. Second, the aldosterone response to AII is potentiated when PKC is maximally translocated to the membrane by a phorbol ester. These data suggest that PKC activation by AII is involved in such functions as cell growth rather than the direct stimulation of aldosterone secretion.

Further studies have been performed to characterize the novel AII binding sites found in the rat and mouse fetus. These receptors are widely distributed throughout skeletal muscle and connective tissue during the last third of fetal life and are reduced by 80% one day after birth. The role of AII in fetal development is under study in secondary cultures of cells prepared from fetal skin. These cells have fibroblast-like characteristics and contain abundant AII receptors. Incubation of the cultures with AII resulted in rapid increase in cytosolic calcium and inositol phosphate formation. The prominent and transient expression of functional AII receptors in the fetus suggest a role of AII during intrauterine development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00035-17 ERRB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Structure and Function of Biologically Active Molecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H.C. Chen	Head, SMSPC	ERRB, NICHD
Others:	J.L. Morell	Research Chemist	ERRB, NICHD
	J.H. Brown	Research Chemist	ERRB, NICHD

COOPERATING UNITS *(if any)*

Department of Clinical Pathology, CC, NIH (C.M. Huang)
Laboratory of Cell Biology, NHLBI, NIH (R.W. Hendler)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Molecular Structure and Protein Chemistry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS.	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During this project year, structural design, chemical synthesis, conformational studies, and the mode of action of the vertebrate peptide antibiotics, magainins, have been studied to allow elucidation of the relationship of alpha-helicity, amphiphilicity and stability to proteases in eliciting broad spectrum antimicrobial activity without causing toxicity to mammalian tissues. Substitution of low propensity residues (Gly13,18 and Ser8) in the natural magainin 2 sequence by Ala enhanced the alpha-helical structure and gave analogues with increases of up to two orders of magnitude in antimicrobial activity and no appreciable increase in hemolytic activity over the natural magainins. Although more than 500 analogues have been synthesized by various laboratories, none surpasses the level of antimicrobial activity of our Ala substituted analogues. Studies with eight magainin 2 amide analogues of D-Ala and L-Ala permuted at positions 9, 13, 18 have revealed that D-Ala modification reduces alpha-helical potential. In evaluating antimicrobial activity, modification at position 13 or 18 is less important than at 9 and the cumulative effect of a D-Ala modification at 9 and 13 is more deleterious than at 13 and 18. These results suggest that helix in the N-terminal region is most important in eliciting biological activity due to the presence of positive charges which can interact with membrane phospholipids. The dissipation of membrane potential is likely the mechanism of action for magainins. We have demonstrated that a potent magainin A analogue (MA) decreased membrane potential and released respiratory control more effectively than magainin 2 amide (M2a) in E. coli cells and cytochrome oxidase liposomes. In the liposome system the uncoupling activity of M2a was reversed in the presence of pronase, whereas the activity of MA was not affected. This observation suggests that MA, which is a strong former of alpha-helix, interacts with lipid much more readily than M2a, and is thereby less susceptible to proteolysis. Such resistance to proteolysis may be in part responsible for the increase in antimicrobial activity of the potent analogues. An 18-residue peptide sequence deduced from the rat prolactin receptor gene was synthesized by the solid phase method. Since the sequence contains three Lys residues, the epsilon amino-trifluoroacetyl (Tfa) peptide was synthesized, conjugated to bovine thyroglobulin and then treated with 2 N piperidine to remove Tfa to give a conjugate suitable for the generation of antisera to the prolactin receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00146-14 ERRB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Structure and Function of Chorionic Gonadotropins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: H.C. Chen Head, SMSPC ERRB, NICHD

Other: J.H. Brown Research Chemist ERRB, NICHD

COOPERATING UNITS *(if any)*

Developmental Endocrinology Branch, NICHD, NIH (L. Liu);
Department of Chemistry, Georgetown University (D.C.H. Yang)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Molecular Structure and Protein Chemistry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project focuses on the role of carbohydrate structures of human chorionic gonadotropin (hCG) in the subunit association kinetics and effect on in vivo biological activity. Re-evaluation of previous studies measuring the fluorescence enhancement of 1-anilino-naphthalene-8-sulfonate (ANS) occurring with hCG subunit association has revealed that the reassociation rates for deglycosylated subunits is 23-fold faster than that of the native hCG molecule. An initial time lag was noted during the reassociation. The fit of the data against four different models was tested and was found to be most consistent with a model which predicts that the order of the reaction and the extent of lag depend on the concentration of the deglycosylated alpha-subunit. The rate limiting step appears to be the conformational transition of the deglycosylated alpha-subunit. Although the conformational transition of beta-subunit may take place as well, the significance of this process as rate determining is minimal in all models tested. We postulate that hCG may be assembled as a deglycosylated form in living cells and the excess secretion of the carbohydrate-rich non-associable free hCG-alpha subunit in disease states may be caused by over-glycosylation of the alpha-subunit.

We have prepared, purified and characterized HF-deglycosylated hCG for the studies of pharmaco-kinetics and stimulation of testosterone production in the Cynomolgus monkey in vivo. These studies show that the removal of carbohydrate structure from hCG causes profound changes in the rate constant of the hormone disappearance without changes in the initial volume of distribution. The deglycosylated hCG also remains a full agonist at the LH receptor in the primate in vivo consistent with our previous findings in in vitro systems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00147-14 ERRB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Action of Peptide Hormones in Steroidogenic Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M.L. Dufau	Head, SME	ERRB, NICHD
Others:	M. Kitamura	Visiting Fellow	ERRB, NICHD
	M. Hirabayashi	Visiting Fellow	ERRB, NICHD
	E. Buczko	Adjunct Scientist	ERRB, NICHD
	C. Winters	Chemist	ERRB, NICHD
	S. Ulisse	Adjunct Scientist	ERRB, NICHD
	A. Fabbri	Adjunct Scientist	ERRB, NICHD
	J. Tinajero	Adjunct Scientist	ERRB, NICHD

COOPERATING UNITS (if any)

Contract for preparation of gonadal cells and cell fractions HD-6-2904

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Molecular Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Luteinizing hormone is the major regulator of Leydig cell differentiation and steroidogenic function. A number of hormones produced by the Leydig cell (e.g. estrogen, angiotensin, CRF, vasopressin) and the tubular compartment (inhibin, TGF beta), can influence both acute and chronic actions of LH. Conversely, hormones produced in the Leydig cells modulate tubular function (e.g. androgen, beta-endorphin, oxytocin). The LH stimulatory event can be negatively influenced by the action of angiotensin II through the guanyl nucleotide inhibitory unit of adenylate cyclase. We have recently discovered an action of corticotrophin releasing hormone through specific high affinity low capacity receptors in the Leydig cells which involves a pertussis toxin insensitive guanyl nucleotide regulatory unit with interaction between signalling pathways and resulting inhibition of LH induced cAMP generation and consequently of steroidogenesis. In contrast to brain, pituitary and other peripheral tissues CRF in the Leydig cell, did not couple to Gs. The CRF inhibitory action in the Leydig cells is exerted through direct or indirect action of protein kinase C, at the level of one or more components of adenylate cyclase.

Physiological increases in endogenous LH action causes positive regulation of membrane receptors and steroidogenesis while major elevations in circulating gonadotropin can induce down-regulation of LH receptors and desensitization of steroid responses in the adult cell. Gonadotropin-induced desensitization in adult rat testes include an estrogen mediated steroidogenic lesion of the microsomal enzymes 17alpha-hydroxylase/17-20 desmolase. For further understanding of the structure and regulation this key enzyme of the androgen pathway, the rat P450-17alpha cDNA was cloned and sequenced. This cDNA expressed in COS-1 cells 17alpha hydroxylase/17,20 desmolase activities. From the deduced aminoacid sequence, two transmembrane regions were identified, a signal peptide for insertion in the ER, and a 2nd transmembrane region separated from the first by 122 amino acids could serve as the stop-transfer sequence. The structure of carboxy terminal non-transmembrane region is composed of 4 hydrophobic clefts where cleft II would contain the putative steroid binding site for both hydroxylase and lyase activities. Gonadotropin stimulation and desensitization of P450-17alpha dependent enzymes (17alpha-hydroxylase/17,20 desmolase) are related to levels of P450-17alpha mRNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00149-14 ERRB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Bioassay of Serum Luteinizing Hormone (LH) and Chorionic Gonadotropin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: M.L. Dufau Head, SME ERRB, NICHD

COOPERATING UNITS *(if any)*

University of Virginia, Dept. of Medicine, Charlottesville, VA (J. Veldhuis)
Contract for preparation of gonadal cells and cell fractions HD-6-2904

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Molecular Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

LH is released from the pituitary gland in pulses of high biological activity. This episodic mode of secretion may obviate gonadotropin-induced desensitization of gonadal cells under hormonal control. The exact nature of glandular secretory events is difficult to discern in vivo, since underlying patterns of hormone release are confounded by metabolic clearance. Using a deconvolution model that allows the calculation of endogenous clearance kinetics and secretory rates simultaneously, multiple parameter deconvolution disclosed endogenous bioactive LH half-life of 53 ± 5.4 min and an endogenous production rate of 0.48 ± 0.06 mIU/ml/min. The bioactive LH secretory burst of half duration of 12.2 ± 1.5 min occurred at intervals of 56 ± 1.3 min and achieved amplitudes of 2.1 ± 0.26 mIU min. LH secretory bursts were positively correlated with the duration of the subsequent interpulse interval. Application of the deconvolutiom algorithm in conjunction with the rat Leydig cell testosterone bioassay revealed the presence of distinct burst-like secretory events acted on by exponential metabolic clearance mechanisms, and provided a good model for analysis of bioactive plasma hormone concentration profiles in vivo. Such multifaceted changes might not be readily apparent using deconvolution techniques requiring known half-lives/or conventional pulse analysis of plasma hormone concentration. The present analysis indicates that endogenous clearance mechanisms provide a mechanism to extend the defined short-lived pituitary secretory events. The multiple parameter deconvolution approach may help to unmask pathophysiological states with alterations in endogenous hormone as well as clearance in vivo. The estrogen time-dependent effects on the kinetics of GnRH's self-priming action on the pituitary gland were analyzed in post-menopausal women on intravaginal estradiol replacement. The self-priming action of GnRH on bioactive LH release is maximal after 5 to 10 days of estrogen exposure and can be observed whether it is defined as increase in the percentage amplitude of a second peak of bioactive LH provoked by exogenous GnRH (10 micrograms,i.v) compared to the first LH peak (given at 2-hourly intervals) or as an incremental increase. We conclude that estrogen in the human regulates the pituitary gland's responsiveness to GnRH, such that enhanced secretion of biologically active gonadotropin occurs during serial exposure to GnRH stimulation. The finding of GnRH self-priming induced by estradiol is of importance to an understanding of possible mechanisms governing the generation of the preovulatory gonadotropin surge.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00150-14 ERRB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Gonadal Prolactin, LH/hCG Receptors and Coupling Functions.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M.L. Dufau	Head, SME	ERRB, NICHD
Others:	C-H. Tsai-Morris	Sr. Staff Fellow	ERRB, NICHD
	Y. Funatsu	Visiting Associate	ERRB, NICHD
	E. Buczko	Adjunct Scientist	ERRB, NICHD
	C. Delgado	NRSA Fellow	ERRB, NICHD
	R. Zhang	Adjunct Scientist	ERRB, NICHD

COOPERATING UNITS (if any)

Contract for preparation of gonadal cells and cell fractions HD-6-2904

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Molecular Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
3.9	3.4	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Nb2 lymphoma cell is dependent upon lactogens for proliferation and provides an invaluable model for the study of prolactin (PRL) receptor coupling functions and messengers for prolactin's multiple biological actions. We previously demonstrated that the proliferative effect of prolactin was modulated by pertussis and cholera toxins and that these effects were independent of the cAMP effects on prolactin-induced mitogenesis, indicating that G-proteins could be involved in signal transduction steps. We also showed that exposure of Nb2 cells to prolactin caused time and dose dependent changes in the ability of specific 38 kDa and 41.5 kDa membrane proteins to be subsequently ADP-ribosylated by pertussis and cholera toxins respectively. These include rapid reduction of the 41.5 kDa substrate to cholera toxin and marked increase of the 38kDa substrate for pertussis toxin. This is the first demonstration of a direct effect of prolactin on G-proteins, suggesting their probable involvement in the signal transduction mechanism of the prolactin receptor leading to proliferation of Nb2 lymphoma cells. The purified ovarian LH/hCG receptor was identified as a monomeric protein (Mr 80,000) and its homogeneity was confirmed by microsequencing. The N-terminal peptide sequence was NH2-R-E-L-S-G-S-R-X-REP-D-X-D-X-A-P-D-G. A sequence derived from cyanogen bromide cleavage (P-L-V-G-I-S-N-Y) was used to raise antibodies and to screen genonic and cDNA libraries. cDNA clone was isolated with 61% overall nucleotide similarity with the recently cloned LH receptor and is now under identification. Since the LH/hCG receptor of testis and ovary is only available in microgram quantities, the use of phosphorylated receptors has facilitated structural studies. In initial studies using reduced phosphorylated receptor, we demonstrated that the LH/hCG receptor contains sialylated N-linked carbohydrate chains of the biantennary type, the hybrid type or both. Complete enzyme digestion (neuraminidase, N-Glycanase) of phosphorylated receptors with conditions for preservation of bindability of blotted native receptor were attained (Mrs from 90-92 kDa to 82 kDa (testis) and 80-85 kDa (ovary) or N-glycanase reduction to 60 kDa in both tissues). Subsequently, by binding of 125I-hCG to blots of desialylated or deglysosylated native receptor from SDS/PAGE, we demonstrated that while the terminal sialic acid of the glycosidic chain would not be involved in hormone binding, the removal of glycosyl residues by N-Glycanase caused a major decrease in hormone binding, indicating the participation of N-linked carbohydrates in agonist-receptor interactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00151-14 ERRB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Gonadal and Placental Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. J. Catt	Head, SHR	ERRB, NICHD
Others:	M. Zilberstein	Research Associate	ERRB, NICHD
	C. Moretti	Adjunct Scientist	ERRB, NICHD
	J. Ohnishi	Guest Researcher	ERRB, NICHD
	N. Solan	Adjunct Technician	ERRB, NICHD

COOPERATING UNITS *(if any)*

Human Genetics Branch, NICHD (J. Chou); Diabetes Branch, NIDDK (D. LeRoith)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Hormonal Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous studies on the molecular basis of hormone action during granulosa cell differentiation included evaluation of the functions and mechanisms of action of pituitary hormones and growth factors. TGF-beta was found to exert bifunctional actions on the maturation of granulosa cells, and to modulate FSH-induced stimulation of cAMP formation, steroidogenesis, and LH receptor expression. TGF-beta also accelerated the maturation of both follicle-enclosed oocytes and cumulus-oocyte complexes, with significant increases in the rate of germinal vesicle breakdown. Other growth factors including IGF-I, IGF-II, and EGF also stimulated germinal vesicle breakdown. For further studies on growth control of rat granulosa cells, which do not undergo division in culture, a continuous cell line was derived by transformation of granulosa cells with an SV40 tsA255 mutant, which has a temperature-sensitive mutation in the gene required for maintenance of transformation. This granulosa cell-derived line (RGA-41S) exhibits temperature-regulated proliferation behavior with unrestrained growth and a transformed phenotype of the permissive temperature (33 °C) and a differentiated non-transformed phenotype with growth arrest at the nonpermissive temperature (40 °C). The cultured RGA-41S cells were found to produce IGF-I mRNA and to secrete IGF-I, and also to express receptors for IGF-I as well as the corresponding mRNA. These cells provide a model system for studies on the paracrine and autocrine actions of IGF-I in the growth and differentiation of epithelial cells.

In normal granulosa cells, studies were commenced on the regulation of the ovarian renin-angiotensin system, employing solution hybridization procedures to measure the steady state levels of renin and angiotensinogen mRNA during maturation and stimulation of granulosa cells by gonadotropins and growth factors in vitro. Also, based on recent findings that growth hormone releasing hormone (GHRH) increases the incidence of ovulation in women undergoing gonadotropin therapy for infertility, and that immunoreactive GHRH is present in the gonads, local actions of GHRH on granulosa cell function were evaluated in vitro. Initial studies revealed consistent effects of GHRH on cyclic AMP production and steroidogenesis in the granulosa cell, revealing a local regulatory mechanism with the potential to synergize with FSH at the ovarian level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00184-11 ERRB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Pituitary Hormone Secretion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. J. Catt	Head, SHR	ERRB, NICHD
Others:	S.-I. Izumi	Visiting Fellow	ERRB, NICHD
	S. Stojilkovic	Visiting Scientist	ERRB, NICHD
	M. Virmani	Adjunct Scientist	ERRB, NICHD
	S. Dufour	Adjunct Scientist	ERRB, NICHD
	R. Omenjaniuk	Adjunct Scientist	ERRB, NICHD
	L. Krsmanovic	Adjunct Scientist	ERRB, NICHD

COOPERATING UNITS *(if any)*

Lab of Cell Biology and Genetics, NIDDK (E. Rojas)
Contract for preparation of adrenal and pituitary cells N01-HD-0-2806

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Hormonal Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pituitary gonadotropin secretion is primarily regulated by GnRH, which binds to and activates specific receptors in the plasma membrane of the gonadotroph. The GnRH receptor was solubilized and shown to exist as a 250 kDa complex under non-denaturing conditions and to contain a 52 kDa binding subunit demonstrable by photo-affinity labeling. GnRH action is expressed through the activation of calcium-phosphoinositide signaling mechanisms, leading to a characteristic biphasic mode of cytosolic calcium elevation and gonadotropin release from the pituitary gland. The initial phase of LH secretion depends largely on mobilization of intracellular calcium, whereas sustained hormone secretion is dependent on entry of extracellular calcium through plasma-membrane channels. In addition to the InsP3-mediated early rise in [Ca]i and LH release, other phospholipid products including diacylglycerol (DG) and arachidonic acid (AA) are involved in cellular activation by GnRH. Studies with enzyme inhibitors revealed that GnRH activates two distinct lipases, DG lipase and phospholipase A2, to produce arachidonic acid. Activation of protein kinase C by DG also participates in GnRH action, in part by mechanisms independent of calcium mobilization, but also by promoting calcium influx through dihydropyridine-insensitive channels. The involvement of protein kinase C in gonadotropin synthesis and secretion was also shown by studies in pituitary cells depleted of protein kinase C, in which the secretory actions of GnRH and phorbol esters were substantially impaired. Studies of the nature of the calcium channels involved in GnRH action revealed the presence of voltage sensitive calcium channels (VSCC) in all five populations of anterior pituitary cells, with an average number of about 1000 channels per cell. Calcium entry through L-type VSCC was found to account for about 50% of the elevated cytosolic calcium and LH secretory response during sustained stimulation by GnRH. Also, inactivation of VSCC during GnRH action was shown to occur by a calcium-dependent mechanism and to be responsible for the onset of desensitization, a prominent feature of GnRH action and an important process in therapeutic suppression of gonadotropin secretion. In addition to rapid regulation by GnRH, gonadotrophs were found to be more slowly activated by neurohypophyseal hormones, acting via a distinct receptor of the oxytocin type, in contrast to the vasopressin-type receptor present in gonadotrophs. This action of oxytocin could provide a complementary hypothalamic control mechanism for long-term modulation of gonadotropin secretion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00187-10 ERRB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Hormonal Regulation of Cellular Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	K.-P. Huang	Head, SMR	ERRB, NICHD
Others:	F. Huang	Expert	ERRB, NICHD
	H. Nakabayashi	Visiting Associate	ERRB, NICHD
	K.H. Chen	IRTA Fellow	ERRB, NICHD
	C.W. Mahoney	NRC Fellow	ERRB, NICHD

COOPERATING UNITS *(if any)*

Lab of Chemical Pharmacology, NHLBI, NIH (M.A. Beaven); Lab of Cell Biology, MH, NIH (W.S. Young); Lab of Mol Oncology, NCI, NIH (T.Y. Shih); Genetic Institute, Cambridge, MA (J.L. Knopf); Centre National De La Recherche Scientifique, Strasbourg, France (A.N. Malviya)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Metabolic Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Phosphorylation-dephosphorylation of proteins is one of the most important mechanisms for the regulation of cellular functions. Protein kinase C (PKC), a Ca2+/phospholipid-dependent protein kinase, has emerged as a pivotal regulatory element for cell growth, differentiation, gene expression, hormone secretion, cell surface receptor function, and cellular metabolism. This protein kinase can be activated by diacylglycerol, a second messenger generated by signal-induced breakdown of phosphoinositides. In addition, it has been identified as a receptor for tumor-promoting phorbol esters which elicit pleiotropic responses comparable to those stimulated by many hormones and growth factors. Three isozymic forms of PKC have been identified from mammalian brains. Polyclonal and monoclonal antibodies against these enzymes were prepared for their immunochemical characterization. These enzymes were found to have distinct tissue, cellular, and subcellular distributions and were differentially expressed during development. The role of PKC in mediating the action of neurotransmitter was investigated with neurons grown in culture. The excitatory amino acid, glutamate, caused a translocation of PKC from cytosol to the particulate fraction. Membrane-association of PKC in the cell is an obligatory step for activation; however, in vitro interaction of PKC with phospholipid resulted in inactivation of the kinase. This inactivation could be partially protected by preincubation with Ca2+ or Mg2+, indicating that these divalent cations interact with the kinase to form an active conformation. The structure/function relationships of the enzyme were investigated by mutagenesis. The mutated PKC cDNAs were expressed in the COS cell and Baculovirus expression systems. Biochemical analysis of the mutated PKCs revealed the region of the molecule important for the binding of phorbol ester. Identification of specific inhibitor of PKCs has a broad application in defining the functional role of these enzymes. We found that suramin, an anti-HIV drug, and a synthetic analogue of magainin, were potent inhibitors of these kinases. The functional roles of the various PKC isozymes in cellular regulation was investigated by selecting mutant cell lines deficient in an isozyme. We have employed several variants of rat hasophic leukemia cells deficient in type II PKC as models to determine the effects of transfection with PKC genes on the secretory response of these cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00191-05 ERRB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Neuroendocrine Regulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. Aguilera	Head, SEP	ERRB, NICHD
Others:	P. Carvallo	Visiting Fellow	ERRB, NICHD
	M. Flores	Guest Researcher	ERRB, NICHD

COOPERATING UNITS *(if any)*

Dept. of Psychiatry, UCSD(R.L. Hauger)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Endocrine Physiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Investigation has focused on the properties and regulation of corticotropin releasing factor (CRF) receptors and the mechanisms of interaction between CRF and other regulators of ACTH secretion, with emphasis on adaptation to stress.

A. CRF receptors and pituitary-adrenal responses during stress. Previous studies have shown that the biphasic ACTH responses to prolonged immobilization are accompanied by decreases in pituitary CRF receptors and hypersensitivity of the pituitary to CRF infusion and a second novel stress. Extension of these studies using a more mild form of stress, intermittent immobilization for 2.5 hr daily, showed complete desensitization of the ACTH response after the first 4 days of immobilization, while plasma glucocorticoids were markedly increased. Despite a decrease in pituitary CRF receptors, ACTH responses to a novel stress, but not to CRF infusion, were enhanced. These responses were similar to those observed in types of human depression in which hypercortisolemia is a characteristic feature. CRF receptors in the intermediate pituitary were unchanged during immobilization stress. In contrast to the changes during immobilization stress, CRF receptors were unaltered in the anterior pituitary during cold stress, but were markedly increased in the intermediate lobe.

B. CRF receptor properties and coupling to cellular function. The binding characteristics of CRF receptors determined in membranes from the neurointermediate lobes were almost identical to those in the anterior pituitary. Analysis of the solubilized cross-linked CRF-receptor complex in different target tissues by gel electrophoresis showed similar properties in both pituitary lobes, with a molecular weight of about 70 kDa. It is known that CRF receptor occupancy results in activation of adenylate cyclase. In addition, experiments showing that pharmacological inhibition of protein kinase C inhibits ACTH release suggest that the CRF receptor may be also linked to a calcium/phospholipid-dependent transduction system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00193-04 ERRB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Angiotensin II Receptors and Activation Mechanisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	K. J. Catt	Head, SHR			ERRB, NICHD
Others:	G. Aguilera	Head, SEP	K. Sandberg	IRTA Fellow	ERRB, NICHD
	A. Clark	Visiting Scientist	A. Baukal	Chemist, GS-12	ERRB, NICHD
	L. Hunyadi	Visiting Fellow	J. Ely	Adjunct Scientist	ERRB, NICHD
	M. Carson	IRTA Fellow	H. Ji	Adjunct Scientist	ERRB, NICHD

COOPERATING UNITS *(if any)*

Lab. of Molecular and Cellular Neurobiology, NINCDS (C. Collins)
Dept. of Physiology, Semmelweiss University Medical School, Budapest (T. Balla)
Contract for preparation of adrenal and pituitary cells ND1-HD-0-2806

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Hormonal Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	5.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The isolation and activation mechanisms of the angiotensin II (AII) receptor were studied in bovine adrenal glomerulosa cells and other target tissues. The adrenal AII receptor was purified by affinity chromatography and microsequenced, and cDNA probes were prepared and applied to screening of an adrenal cDNA library. A further approach to cloning the AII receptor was initiated by screening mRNA, prepared from an adrenal cDNA expression library, in the Xenopus oocyte. The detection systems for this approach were based on the measurement of electrophysiological and light responses (measured by aequorin) in oocytes injected with mRNA from adrenal extracts and an expression library. The aequorin system was established and validated by measurement of the light responses elucidated by ligand-induced calcium mobilization in Xenopus oocytes injected with poly (A)+ mRNA from rat adrenal cortex and brain, with stimulation by AII, acetylcholine and glutamate. In cultured bovine adrenal glomerulosa cells the rapid effect of AII on Ins-1,4,5-P3 formation was accompanied by a concomitant elevation of cytosolic calcium during the first minute of stimulation, with peaks in both responses at 5-10 sec. The subsequent plateau in calcium was associated with secondary increases in Ins-1,4,5-P3 and Ins-1,3,4,5-P4, suggesting a role of inositol phosphates in maintenance of the prolonged calcium response to agonist stimulation. Extending our previous studies on the production and metabolism of inositol phosphates in AII-stimulated adrenal glomerulosa cells, the conversion of Ins-1,3,4,6-P4 to InsP5 was demonstrated in bovine adrenal cytosol and permeabilized bovine glomerulosa cells, establishing Ins-1,3,4,6-P4 as a link between InsP3 metabolism and the higher inositol phosphates recently described in the adrenal gland and other mammalian tissues. In addition to the conversion of Ins-1,3,4,6-P4 to InsP5, two other InsP4 isomers were detected and characterized in AII-stimulated adrenal cells. These were identified as Ins-3,4,5,6-P4, shown to be an additional precursor of InsP5, and its stereoisomer, Ins-1,4,5,6-P4, shown to be formed by dephosphorylation of InsP5. AII stimulation was found to cause a short-term increase in conversion of InsP5 to Ins-1,4,5,6-P4, but a long-term elevation of Ins-3,4,5,6-P4 and InsP5, thus establishing a connection between agonist-stimulated phosphoinositide hydrolysis and the production of higher inositol polyphosphates in the adrenal glomerulosa cell.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00194-01 ERRB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

ACTH Regulation of Adrenocortical Function (Steroidogenesis): Biochemistry and Molecular Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	C.A. Strott	Head, SACB	ERRB, NICHD
Others:	Y.C. Lee	Sr. Staff Fellow	ERRB, NICHD
	W.J. Driscoll	IRTA	ERRB, NICHD
	T. Demura	Visiting Fellow	ERRB, NICHD

COOPERATING UNITS *(if any)*

Section on Molecular Structure and Protein Chemistry, NICHD (Dr. H.C. Chen)
Laboratory of Neurochemistry, NINDS (Dr. Mark Whitnall)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Adrenal Cell Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The steroidogenic response to ACTH can be separated into acute and subacute aspects. The acute response(sec-min) occurs primarily at the level of mitochondria and involves the translocation of cholesterol to the inner mitochondrial membrane followed by the conversion of cholesterol to pregnenolone (rate-limiting). The subacute response (hours) appears to occur at the level of the genome and involves synthesis of enzymes and co-factors. Both the acute and subacute responses to ACTH are mediated by cAMP and cAMP-dependent protein kinase, and are dependent on the synthesis of protein. An adrenocortical steroidogenic regulatory protein has been proposed but none has been identified as yet. The role of Ca2+ in ACTH action is complex; Ca2+-regulated protein kinases may be involved. Thus, the molecular mechanisms involved in ACTH action remain to be elucidated. Pregnenolone, the product of the rate-limiting step in steroidogenesis, is poorly soluble in aqueous media and must be translocated between intracellular compartments; the translocation process is not understood. Furthermore, a biological role for pregnenolone other than that of a precursor has not been established. Progesterone, which is derived from pregnenolone and closely related, not only serves as a key steroid intermediate itself but is a ligand for a member of the steroid receptor super gene family. To examine the complex process of ACTH action, the possible involvement of specific steroid-binding proteins has been explored. A 34 kDa cytosolic pregnenolone-binding protein (P5BP) has been identified, purified and examined. The P5BP has multiple forms with PI's of 6.5, 5.5, 5.4 and 5.2. Individual isoforms have been isolated, antisera generated and immunological identity demonstrated. Pregnenolone-binding activity is abolished by treatment with alkaline phosphatase. The P5BP isoform patterns in the outer ACTH-responsive and inner ACTH-unresponsive zones are distinct and markedly altered by phosphatase treatment. The N-terminus of P5BP is blocked necessitating determination of internal peptide sequences after partial tryptic digestion. A 75-79 kDa acidic nuclear progesterone-binding protein (P4BP) has been identified and purified. Biochemical and immunological evidence indicate that P4PB is distinct from the classical progesterone receptor. Specific antibodies and synthetic oligonucleotide probes for both the P5BP and P4BP will be used to screen a guinea pig adrenocortical cDNA library. In addition, a tissue culture procedure is being developed to systematically examine the regulation and function of both binding proteins.

HUMAN GENETICS BRANCH (HGB)

ZO1 HD 00131-15	Human Biochemical Genetics William A. Gahl, M.D., Ph.D.
Z01 HD 00133-12	Study of Glycogen Storage Disease James B. Sidbury, Jr., M.D.
Z01 HD 00137-15	Regulation and Expression of the UDP Glucuronosyltransferase Gene Family Ida S. Owens, Ph.D.
Z01 HD 00404-07	Cell and Sulfur Metabolism in Fibroblasts of Genetic Diseases Jean DeB. Butler, Ph.D.
Z01 HD 00408-06	Pathophysiology and Treatment of Human Genetic Diseases Joan C. Marini, M.D., Ph.D.
Z01 HD 00410-04	Metabolism in Children with Glycogen Storage Disease, Type I James B. Sidbury, M.D.
ZO1 HD 00412-02	Molecular Regulation of Gene Expression Samuel Adeniyi-Jones, M.D., Ph.D.
Z01 HD 00909-10	Fetal Alcohol Syndrome Anil B. Mukherjee, M.D., Ph.D.
ZO1 HD 00910-10	Biochemistry, Molecular Biology, and Physiology of Phospholipase A_2 Inhibitory Proteins Anil B. Mukherjee, M.D., Ph.D.
Z01 HD 00912-10	Gene Regulation and Cellular Differentiation Janice Y. Chou, Ph.D.

Human Genetics Branch

The Human Genetics Branch conducts both clinical and basic research programs directed toward elucidating the causes and optimal treatments of human genetic diseases. Clinical research projects focus largely upon the inborn errors of metabolism and other heritable disorders of man. Particular emphasis is placed on determining the natural history, diagnosis, and innovative therapy of rare diseases of children. The basic research programs of the Human Genetics Branch provide a foundation for the clinical research endeavors. Bench work employs the use of in vitro systems to study cell biology with special emphasis on models of human development and genetic disease. One Section and one Unit in the Branch have dual responsibilities for patient care as well as basic research; these are the Section on Human Biochemical Genetics and the Unit on Connective Tissue Disorders.

The Section on Human Biochemical Genetics, under the direction of William A. Gahl, studies rare inborn errors of metabolism from the patient to the bench. A great deal of effort is invested in the care and investigation of children and young adults with nephropathic cystinosis. This autosomal recessively inherited disorder results from defective transport of the disulfide amino acid cystine out of lysosomes and into the cytoplasm of cells. The accumulation and crystallization of cystine destroys several tissues, and classically causes death from renal failure by ten years of age. Renal transplantation resolves the kidney problem, but continued cystine accumulation progressively destroys other organs. Therapy is directed at reducing the cellular cystine content. This is accomplished by administration of oral cysteamine, an aminothiol whose cystine-depleting effects have proven efficacy in improving growth and maintaining renal glomerular function.

The Section on Human Biochemical Genetics cares for one third of the 200 patients in the United States and serves as a national referral and international resource center regarding this disease. Pre-transplant patients receive oral cysteamine (or the more palatable phosphocysteamine) in standard-dose or in high-dose, as part of a national clinical trial to determine the optimal dosage regimen of this drug. The Section on Human Biochemical Genetics is the only group in the country to offer cysteamine to post-transplant patients as well.

Infants with cystinosis have renal tubular Fanconi syndrome, in which small molecules fail to be reabsorbed by the kidney and are excreted in the urine. One of the issues in cystinosis treatment is whether early administration of oral cysteamine can prevent the Fanconi syndrome. We have treated one patient with cysteamine from 14 days of age; this boy is now 24 months old with a very mild Fanconi syndrome and remarkably good growth compared to that of his older affected brother in whom treatment was begun later in life. We have also improved the treatment of Fanconi syndrome itself by providing oral L-carnitine supplementation (at 100-200 mg/kg/day) to affected children, whose kidneys fail to reabsorb L-carnitine and, therefore, have low plasma and muscle levels of this compound. L-carnitine is essential for the transport of fatty acids into the mitochondria for subsequent oxidation and energy production. The Section is conducting a long-term trial of L-carnitine supplementation to determine whether muscle carnitine can be repleted and muscle strength and integrity improved by five years of replacement therapy.

Patients with cystinosis develop cystine crystals in their corneas by approximately one year of age. These crystals are refractory to oral cysteamine therapy, but we have demonstrated that 0.1% (10 mM) cysteamine eyedrops, given hourly-while-awake, can dissolve these crystals in very young children. Older patients, with more crystals, were poorly responsive. Therefore, in conjunction with Dr. Kaiser-Kupfer of the National Eye Institute, and after demonstrating the range of toxicity of topical cysteamine in rabbit eyes, we increased to 0.5% the concentration of cysteamine eyedrops administered to cystinosis patients. We now have seven children age 2-5 years who have objective evidence of clearing of corneal crystals. This may prove of long-term benefit to these patients, because the crystals eventually become so packed that they produce a hazy cornea and recurrent corneal erosions. Cysteamine eyedrop therapy is now being extended to include children who have even more densely-packed corneas.

Members of the Section on Human Biochemical Genetics have also recognized the occurrence of serious non-renal complications in cystinosis. In our care of 30 post-renal transplant patients, we have discovered a cystinosis myopathy which involves muscle weakness and atrophy and the risk of aspiration. In conjunction with Dr. Barbara Sonies of the Rehabilitation Medicine Department, we have discovered that the majority of patients over ten years of age have swallowing abnormalities. We also reported several patients with cerebral atrophy on CT scan and three patients who have paraventricular and basal ganglia calcifications. In the past year, we reported the first case of pregnancy in a cystinosis woman; she delivered an entirely normal male infant despite having cystine crystals in the maternal, but not the fetal, portion of her placenta. We also found that virtually all cystinosis patients have delayed puberty, and males have primary hypogonadism and aspermia. This work was done in conjunction with Drs. Chik and Merriam of the Developmental Endocrinology Branch of NICHD. All these efforts at defining the natural history of cystinosis provide the first steps in our efforts to better care for patients with this multisystemic disease.

The Section has taken a recent interest in the study of the neurological, renal and ophthalmic complications of Lowe (oculocerebrorenal) syndrome. Within the past year, Dr. Lawrence Charnas has studied 25 males with this X-linked disorder and reported variable degrees of central demyelination, detected by CT and MRI scans, in these patients. He has also found three patients who had dermoid tumors of unknown etiology. All of the patients displayed marked proteinuria, with a characteristic pattern distinguished by prominent bands in the gamma region on urine protein electrophoresis. The particular type of Fanconi syndrome in Lowe syndrome patients is being defined as primarily proteinuria with a mild to moderate loss of small molecules such as phosphate, amino acids, and carnitine. The recruitment of patients to the NICHD allows us to define the natural history and to assist in the treatment of Lowe syndrome patients, but it also provides material for the investigation of the basic defect in this disease. This is being approached through measurement of nucleotide pyrophosphatase, an enzyme reported elevated in Lowe syndrome fibroblasts. The patient population also provides DNA for future molecular biology investigations into the Lowe syndrome gene, which is located on Xq 24-26. Family studies are also being performed since lenticular opacites have been identified in several obligate heterozygotes for the disease.

Members of the Section have reported the results of treating 5 pyridoxine-nonresponsive homocystinuria patients with oral betaine at 3 grams b.i.d. A two year, double-blind, placebo-controlled crossover study showed that betaine, which aids in remethylating homocysteine to methionine, did not improve bone density in the homocystinurics.

Other work by Dr. Henk Blom, a Visiting Fellow in the lab, showed that a product of methionine transamination, dimethylsulfide, was elevated in patients with methionine adenosyltransferase deficiency, as well as in a boy with hypermethioninemia due to cystathionine β-synthase deficiency. This establishes the existence of the transamination pathway in methionine metabolism.

Members of the Section have diagnosed a 21 month-old boy with severe liver disease and liver copper storage as having Indian Childhood Cirrhosis (ICC). This is the second case of ICC reported in the United States. The findings of electron-dense aggregates in cultured fibroblasts and consanguinity in the family indicate the genetic nature of this defect, which was previously considered to be environmental and confined to India.

Members of the Human Genetics Branch also received fetal blood samples obtained by cordocentesis from Dr. Nicolaides of King's College in London. Analysis of plasma amino acids in these samples by Isa Bernardini established normal values for fetuses at different gestational ages. This will prove valuable for future diagnosis of aminoacidopathies by cordocentesis. Bernardini also found that six amino acids, i.e., lysine, valine, threonine, methionine, tyrosine, and phenylalanine, were concentrated by the human fetus, while nonessential amino acids were not. Some of the blood samples were from intrauterine growth retarded fetuses, and five of these samples failed to concentrate even the essential amino acids mentioned above. It was also determined that carnitine is not concentrated by the normal fetal circulation.

The Section on Human Biochemical Genetics has not limited itself to clinical work. Members of the Section study sialic acid metabolism from a standpoint of various human mutations. One of these is infantile free sialic acid storage disease (ISSD), a rare lysosomal disorder in which free sialic acid accumulates within lysosomes. ISSD fibroblasts were found to store within their lysosomes another charged sugar besides sialic acid, namely, glucoronic acid. This suggests that both glucoronic acid and sialic acid are transported by the same carrier known to be defective in ISSD. Members of the Section also had access to two cell strains in which sialic acid accumulated in the cytosol rather than the lysosomes. These patients have sialuria, and in collaboration with Drs. Gil Ashwell, Peretz Weiss, and Frank Tietze of the NIDDK, it was determined that this disorder is caused by a failure of CMP-sialic acid to feedback inhibit the initial, regulatory enzyme in sialic acid synthesis, UPD-N-acetylglucosamine 2-epimerase. This is an extremely rare inborn error which is due to defective allosteric inhibition with preservation of the catalytic activity of the enzyme. The Section has also studied patients with sialic acid disorders to determine how the human kidney handles sialic acid. In fact, this charged sugar is filtered but not reabsorbed by the human kidney.

The Section continues its investigation into lysosomal transport of small molecules. One specific pursuit is the study of system h for tyrosine and neutral amino acids which was characterized in rat FRTL-5 thyroid cells. Tyrosine, phenylalanine, and leucine transport were each stimulated 3 to 7-fold by thyroid-stimulating hormone (TSH) in the system. This makes system h the first integral lysosomal membrane carrier shown to be hormonally responsive. System h also carries monoiodotyrosine (MIT) across the lysosomal membrane. MIT is produced by lysosomal hydrolysis of thyroglobulin. The iodine is salvaged in the cytosol for reutilization and this requires transport of MIT across the lysosomal membrane. The MIT carrier is also stimulated by TSH and appears to recognize diiodotyrosine (DIT). This allows the use of ^{125}I-DIT as a natural photoaffinity label which binds covalently to molecules in its vicinity upon exposure to ultraviolet light. In collaboration with Dr. Len Kohn of the NIDDK, ^{125}I-

DIT was found to bind to a 70 Kd protein whose N-terminus was sequenced. A 53-mer candidate oligonucleotide was synthesized and served as a probe against an FRTL-5 library. Positive colonies are now being screened in a procedure that may help isolate the gene for the lysosomal MIT/DIT carrier. A primary goal of the Section is to isolate a functional integral lysosomal membrane protein, e.g., a carrier protein.

In a separate project, Jean Butler is studying a mutant mouse with a defect in cholesterol esterification and with lysosomal accumulation of cholesterol. This mouse resembles Niemann-Pick C disease, as studied in human fibroblasts, which also store large amounts of cholesterol in their lysosomes and Golgi complexes. Dr. Butler and her collaborators have shown that HMG CoA reductase, a rate limiting enzyme for cholesterol anabolism, and receptor-mediated LDL uptake are normal in Niemann-Pick C cells. Investigation of these cells and the mutant mouse model may help elucidate the pathway of cholesterol movement within cells and identify the defect in Niemann-Pick C disease.

The second organization within the Human Genetics Branch with both patient care and basic research responsibilities is Dr. Joan C. Marini's Unit on Connective Tissue Disorders. Dr. Marini studies the molecular basis of heritable disorders of connective tissue and their treatment. The Unit focuses its efforts on osteogenesis imperfecta (OI), a generalized connective tissue disorder whose hallmark is a tendency to easy bone fractures. OI has also been associated with defects in the structure and function of type I collagen, a major protein of bone and skin. Dr. Marini cares for a total of 50 children with OI under two clinical protocols. One protocol enrolls 25 children and involves the evaluation of endocrine parameters of growth failure in OI. This study has produced the only known description of the function of the growth hormone axis in a primary bone disorder; the data are now being compared to those of normal children generated by the Developmental Endocrinology Branch of NICHD. In another protocol, in collaboration with the Rehabilitation Medicine Service of the Clinical Center, the Unit continues to study the effect of lower limb bracing on ambulation in children with moderately severe OI.

In the area of basic research, the Unit has developed a system for the detection of point mutations in type I collagen mRNA by RNase A digestion of mismatches in RNA/RNA hybrids between anti-sense riboprobes and the mRNA of patients. This anti-sense riboprobe system now covers the entire mRNA for the two genes coding for type I collagen. The Unit has also developed a complementary methodology for the rapid isolation and sequencing of point mutations which have been detected by the RNA/RNA hybrid system. This approach utilizes amplification of the target region by the polymerase chain reaction (PCR). The patient's poly (A+)-RNA is used for the first strand synthesis of cDNA. This DNA/RNA hybrid is used directly as a substrate for PCR. After amplification of the mismatch-containing region, the amplified fragments are subcloned into appropriate vectors and sequenced.

The riboprobe system has been applied to the mRNA of patients with osteogenesis imperfecta and mismatches have been detected in patients with Types II, III and IV OI. The point mutations have been isolated and sequenced in a case of moderate type IV OI and a case of lethal type II OI. In each case, the mRNA data are in full agreement with the collagen protein data on the patient. In the Type IV case, a single nucleotide change, G→A, resulted in the substitution of a serine for a glycine in amino acid residue 832 of one allele of the α1 (I) chain. In the Type II case, a G→A change resulted in the substitution of an aspartic acid for a glycine at amino acid residue 805 of one allele of the α2(I) chain. Previous models had suggested that substitutions for glycine in the α1(I) chain would be lethal while those in the α2(I) chain would cause a

non-lethal phenotype. This data extends our understanding of the molecular basis of OI and requires alteration of existing models.

The Unit continues to investigate the molecular analysis of patients with connective tissue disorders. Additional mutations in type I collagen of OI patients will be isolated and sequenced, including an unusual case of dominant Type III OI. The Unit also plans to initiate an RNA/RNA hybrid system for Type III collagen and use it to study the molecular defects in patients with Ehlers-Danlos syndrome.

Dr. James B. Sidbury, an Adjunct Scientist in the Human Genetics Branch, continues his studies of glucose metabolism in patients with glycogen storage disease. Adult patients with type I glycogen storage disease due to glucose-6-phosphatase deficiency have been shown to produce glucose at a rate approximating that of normal fasting adults. Dr. Sidbury is also investigating which regimen of raw starch administration will maintain the best and longest blood glucose levels in patients with glycogen storage disease.

In the area of basic research, Dr. Janice Chou leads the Section on Cellular Differentiation in its efforts to understand the regulation of gene expression during normal and abnormal differentiation. The major areas of emphasis are: expression of the α-fetoprotein (AFP) gene in liver; hormonal regulation of liver genes in differentiated hepatocyte lines; establishing mouse liver cells carrying genetic mutations; cloning and expression of the human pregnancy-specific β_1-glycoprotein (PSβG) gene; and cloning and expression of the human alkaline phosphatase (AP) gene.

Over the past several years, the Section has studied expression of the AFP gene in fetal and adult rat livers and in temperature-sensitive hepatocyte lines established by this group. They found that adult rat livers express three AFP mRNAs of 2.2 (minor), 1.7 and 1.5 kb, whereas fetal livers express a 2.2-kb AFP mRNA. A similar 1.7-kb AFP mRNA is also expressed by the SV40-transformed fetal and adult hepatocyte lines and several hepatoma cell lines. The structure of the 1.7-kb transcript was determined by the isolation and characterization of cDNA clones encoding the 1.7-kb variant AFP from cDNA libraries of adult rat liver and a RLA209-15 fetal hepatocyte line. Sequence analysis indicated that the 2.2-and 1.7-kb AFP mRNAs share 1.1-kb of 3' sequences, but they differ in sequences at the 5' regions. The 5' exon (V) of the 1.7-kb mRNA which is absent from the 2.2-kb AFP mRNA is located in the seventh intron of the rat AFP gene. Since there is only one AFP gene per haploid genome in rat, these results suggests that the two transcripts evolved by the alternative use of promoters.

The group also demonstrated that alkaline phosphatase (AP) in human choriocarcinoma cells (malignant trophoblasts) differs from placental AP by its greater sensitivity to EDTA and L-leucine inhibition and its faster electrophoretic mobility on polyacrylamide gel. They found that choriocarcinoma cells express a 2.6-kb AP mRNA unlike normal human placenta, which expresses a 2.8-kb AP mRNA. Administration of sodium butyrate to choriocarcinoma cells greatly increased the steady-state levels of the 2.6-kb choriocarcinoma AP mRNA, resulting in increased enzyme activity and biosynthesis. S1 nuclease analysis, using probes derived from a placental AP cDNA, and ribonuclease protection assays, employing probes derived from the germ-cell AP gene, demonstrated that choriocarcinoma cells express the germ-cell AP gene. The germ-cell AP gene encodes the placental AP-like isozyme which is primarily expressed in the thymus, testis, and germ-cell tumors. The structures of the internal exons (II through X) of the germ-cell AP gene were determined previously based on their similarity to the placental AP gene. However, the boundaries of exons I and XI (3' exon) of the germ-cell AP gene were not defined due to sequence divergence between the two genes in

the 5' and 3' regions. Using the ribonuclease protection and primer extension assays, Chou and coworkers demonstrated that exon I of this gene is 119 bp in length and that germ-cell AP mRNA contains one major transcription initiation site. In addition, they isolated and characterized germ-cell AP cDNA clones from a butyrate-treated choriocarcinoma cDNA library and showed that the germ-cell AP mRNA is 2,487 b in length and that exon XI of this gene is 1135 bp long.

Chou and coworkers have isolated and characterized four distinct cDNA clones encoding human pregnancy-specific β_1-glycoprotein (PSβG). Two types of PSβGs are encoded by these cDNAs: those that contain two repeated protein domains (1a and 2a) and those that contain only one protein domain. All the encoded PSβGs so far analyzed differ in sequence at the carboxyl terminus, indicating that PSβG is a very polymorphic protein. Chou's group confirmed this by identifying multiple PSβG genes in the human genome. They also showed that PSβG shows strong sequence homology to human carcinoembryonic antigen (CEA) which, along with PSβG, belongs to the immunoglobulin supergene family. CEA has been shown to be an intercellular adhesion molecule. Chou and coworkers are currently designing experiments to investigate the possible roles of PSβG as an intercellular adhesion molecule.

The Section has also demonstrated that human placental fibroblasts produce a PSβG immunologically indistinguishable from placental PSβG. They subsequently confirmed this finding by the immunocytochemical localization of PSβG in these fibroblasts. Chou and coworkers found that the major PSβG species synthesized by placental fibroblasts is a 62K glycopolypeptide, whereas the PSβG species found in human placenta consists of one major 72K glycoprotein and two minor 64K and 54K glycoproteins. Thus, the major PSβG species found in fibroblasts and human placenta differ. Synthesis of the 62K PSβG species by fibroblasts was slightly stimulated by sodium butyrate. However, butyrate-treated fibroblasts produced greatly increased amounts of 48K and 72K PSβGs. The similarity in PSβGs produced by butyrate-treated fibroblasts and placenta was confirmed by cell-free synthesis of PSβG. Chou's group previously isolated three PSβG cDNA clones (PSG16, PSG93, and PSG95) which share similar sequences in the 5' region (designated PSG-5'), but differ in sequences in the 3' regions. PSG16 and PSG93 differ only in that PSG93 contains an additional 86-bp sequence at nucleotide 1308 of PSG16. PSG95 contains a 3' untranslated region which differs from that of PSG16/PSG93. Probes derived from these cDNAs hybridized with three PSβG mRNAs of 2.3-, 2.2-, and 1.7-kb in placental fibroblasts, and sodium butyrate increased the steady-state levels of all three mRNAs. In butyrate-treated fibroblasts the PSβG mRNAs that contain the PSG-5' or PSG93-specific sequence are increased to approximately 20% of the levels in human placenta. However, unlike human term placenta which expresses predominantly PSβG mRNAs with 3'-sequences similar to PSG16/PSG93, the butyrate-treated fibroblasts express roughly equal levels of PSβG mRNAs with 3' ends of PSG16/PSG93 and PSG95. Control fibroblasts mainly express a placental PSβG mRNA that shares part of PSG93-specific sequences. This species was only slightly enhanced by butyrate, and therefore, it may be the mRNA that encodes the 62K fibroblast PSβG. The finding that a variant PSβG species is produced in placental fibroblasts suggests that the authentic placental PSβG species may have different functions.

Chou and coworkers have also examined the expression of albumin, tyrosine aminotransferase (TAT), and phosphoenolpyruvate carboxykinase (PEPCK) genes in an adult rat hepatocyte cell line, RALA255-10G, which is temperature sensitive and dependent upon glucocorticoid hormone for differentiation. They found that expression of albumin and TAT genes requires the continued presence of glucocorticoid hormone and this expression was enhanced by cAMP. cAMP alone did not enhance albumin or TAT synthesis. Expression of the PEPCK gene was enhanced by cAMP and

glucocorticoid hormone. Administration of retinoic acid inhibited the glucocorticoid-mediated induction of both albumin and TAT genes, but stimulated the cAMP/glucocorticoid-mediated induction of the PEPCK gene. Transcriptional regulation was the primary site of action. This cell line, established by Chou's group, provides an excellent model to examine mechanisms regulating liver differentiation by glucocorticoids, cAMP, and retinoic acid.

Chou and coworkers have recently established mouse liver cell lines carrying deletions near the albino locus on chromosome 7. These mouse mutants express low levels of several liver-specific genes including PEPCK and TAT. It was proposed that a trans-acting control function localized on chromosome 7 near the albino locus is required for expression of many liver genes. These cell lines were established by transforming primary liver cells with temperature-sensitive A (tsA) mutants of SV40; these are temperature sensitive in the gene required for the maintenance of transformation. The mouse liver cell lines that are ts for growth and differentiation express a variety of mouse liver genes including albumin, transferrin, TAT, and PEPCK. In contrast to in vivo studies which showed that the albino deletion mutant mouse expresses low levels of TAT and PEPCK genes which are insensitive to glucocorticoid and cAMP induction, mouse liver cell lines carrying the albino deletion express both TAT and PEPCK genes and the expression is greatly increased by glucocorticoid hormone and cAMP. These findings indicate that the trans-acting factor which is involved in control of liver gene expression in the albino deletion mouse mutants may not be located in chromosome 7. Alternatively, control of liver gene expression may involve the interaction of multiple factors including the trans-acting factor encoded by chromosome 7 which can not be studied in an isolated tissue.

The Section on Developmental Genetics, under Anil B. Mukherjee, conducts both basic and clinically related research into the biochemistry, molecular biology and genetic regulation of the enzyme phospholipase A_2 (PLA_2) and its inhibitory proteins with antiinflammatory/immunomudulatory properties.

Inflammatory diseases are a major and important cause of human morbidity and mortality and even some presumably non-inflammatory diseases have an inflammatory component. Although excessive inflammation contributes to many disease processes, absence of this phenomenon may lead to a compromised host. Thus, inflammation is essential, ongoing, and a precisely regulated process in a healthy individual.

One of the pathways of the initiation of an inflammatory process is the activation of a lipolytic enzyme PLA_2. This enzyme may also be involved in many physiological functions including cellular signal transduction and immunological activation. PLA_2 is a key enzyme in the production of arachidonic acid from cell membrane phospholipids and plays a regulatory role in the production of eicosanoids, e.g., prostaglandins, leukotrienes and thromboxanes. Some of these eicosanoids are known mediators of inflammation.

For the past eleven years this group has been engaged in investigations which led to the discovery that a steroid-dependent, small molecular weight secretory protein, uteroglobin (UG) is an inhibitor of PLA_2. Last year the group identified two nonapeptides, MQMKKVLDS from UG and HDMNKVLDL from lipocortin-1, which are potent PLA_2 inhibitors and antiinflammatory agents. These oligopeptides appear to represent the active site or a portion of the active site responsible for the PLA_2 inhibitory activity of the proteins from which they are derived. To demonstrate this more conclusively, the group will perform site-directed mutagenesis involving large scale expression of this protein in *E. coli*. However, this poses a unique problem since

the protein is a dimer with two interchain disulfide bridges connecting the monomers; to date, it has not been possible to express a eukaryotic protein containing two disulfide bridges in a bacterial host. To obviate this problem, the Section has successfully designed an expression vector, pLE103-1, in which the expression of recombinant UG is controlled by a bacteriophage T7 late promoter. With pLE103-1, recombinant UG production reached approximately 9-11% of total bacterial soluble proteins. This recombinant protein has been purified to homogeneity. Its N-terminal amino acid sequence confirmed that the protein is identical to natural rabbit UG and formed the disulfide bridges at appropriate amino acid residues, i.e., Cys 3' to 69 and 3 to 69' residues. The recombinant UG was also found to be as active a PLA_2 inhibitor and an antihrombotic agent as its natural counterpart.

The successful development of this expression system is now being used for site-directed mutagenesis studies. Because of its potential biomedical and commercial possibilities, a patent application has been filed on this vector.

The Section has also used a monospecific polyclonal antibody to rabbit UG to screen human lung and prostatic cDNA libraries (λgt11). This yielded several positive plaques. Plasmids from one of these plaques were partially characterized and found to contain a 500 bp human cDNA which is now being sequenced. If this fragment consists of a unique sequence, the group would be able to clone a human UG cDNA.

In collaboration with Dr. David Bullock, the group has generated transgenic mice by pronuclear microinjection of cloned genomic UG DNA including a 2.3 kb of 5'-flanking sequence. UG-mRNA was detected in the lung and the uterus but not in liver, kidney, brain or spleen of transgenic mice. The level of expression of UG-mRNA in transgenic mice lungs exceeded that in the rabbit while uterine expression was weaker. Strong immunofluorescence was present in the bronchial and pulmonary alveolar epithelium but not detectable in the endometrium. Since UG has profound antiinflammatory properties, these mice may provide a valuable model for studies of pulmonary inflammation.

The antiinflammatory peptides described last year (e.g., MQMKKVLDS and HDMNKVLDL) have a shared essential sequence KVLD (P4). This tetrapeptide has no anti-PLA_2 or antiinflammatory activity. However, it does have potent inhibitory activity on platelet aggregation induced by ADP (IC_{50} = 3-6 x 10^{-4}M) and thrombin (IC_{50} = 6.8-7.4 X 10^{-4}M). Although MQMKKVLDS (P1) and P4 inhibit thrombin esterolytic activity, aggregation induced by thrombin was inhibited less effectively than aggregation induced by ADP. Serotonin secretion by platelets was not affected despite inhibition of aggregation. This indicated that the most significant mechanism of inhibition involves a step subsequent to full platelet activation. Platelet binding of ^{125}I-fibrinogen was found to be inhibited by KVLD (K_i = 9 x 10^{-4}M). When P1 and P4 peptides were used together there was an additive effect on inhibition of aggregation. This may suggest that there are two mechanisms involved, e.g., P1 works by inhibiting PLA_2, a necessary step in the activation of platelets, and P4 works by blocking of sites on platelets for fibrinogen binding.

Factors regulating PLA_2 are also being studied in order to understand many cellular events which are as yet unclarified. Recently, this group has described two nonapeptides, derived from uteroglobin and lipocortin I respectively which are potent inhibitors of PLA_2 *in vitro* (Nature 335: 726-730, 1988). They have now identified a novel mechanism of activation of PLA_2 by tissue and plasma transglutaminase (TG). They found that: a) both plasma and tissue (guinea pig liver) TG activate porcine pancreatic PLA_2 *in vitro*; b) this activation is both time and dose dependent and c) the

addition of polyamine substrates of TG in micromolar concentrations significantly reduces the activation. Maximal stimulation (PLA_2 activity 400% of control) was obtained at concentrations of 9 x 10^{-2} units/ml of guinea pig liver TG and with 200 μg/ml of rabbit plasma TG (coagulation factor XIII, preactivated with thrombin). Time courses of the PLA_2 reaction, analyzed by the integrated form of the Michaelis-Menten equation, show that the apparent K_m of PLA_2 is not changed, while the apparent V_{max} is increased by TG treatment. Since it is known that dimerization and aggregation of PLA_2 result in increased enzymatic activity, the action of TG may represent a novel mechanism by which activated forms of PLA_2 can be systematically stabilized. These data suggest that a novel post-translational modification of PLA_2 could play a role in pathological processes such as inflammation, thrombosis and disseminated intravascular coagulation (DIC).

A collaborative clinical study to evaluate arachidonic acid metabolism in cystic fibrosis is ongoing and the investigations on a possible genetic defect in fetal alcohol syndrome is also being continued.

The Section on Drug Biotransformation, under the direction of Ida S. Owens, Ph.D., studies the regulation of the UDP-glucuronosyltransferase gene family at the molecular level using both mouse and human systems. Since an undetermined number of transferase isozymes is involved in detoxifying numerous endogenous and exogenous lipophiles through glucuronidation, the aim of this research program is to isolate and characterize the cDNAs encoding the many isozyme activities including the one for the critical metabolite, bilirubin. A mouse cDNA, UDPGT m-1, has been characterized and shown to encode a protein of 51 kDa which is glycosylated through an asparagine linkage. This isozyme catalyzes a broad range of substrates when expressed in yeast Saccharomyces cerevisiae (AH_{22}) using the vector pEVPII. The compounds include three androgens, an estrogen, three aromatic and two with both aromatic and saturated-ring structures. When the nucleotide sequences encoding the membrane-targeting signal peptide were removed and the 5' end was adapted with a new AUG translation-start site, the protein was located in the cytosol rather than the membrane. The catalytic activity of the cytosolic form is very much reduced for all substrates except naphthol, which is preferred by the membrane-bound version of the enzyme.

While using UDPGT m-1 as a probe, a human liver cDNA, UDPGT h-1 has been isolated and characterized. It is 2000-bp in length and is encoded in three different sized mRNAs, 2.3, 3.6, and 5.3-kb attributable to corresponding increments in lengths of 3' untranslated extensions. The 2.3-kb species is highly abundant, while the 5.3-kb species is barely detectable. The cDNA inserted into pEVPII and expressed in AH_{22} cells expresses a 51-52 kDa protein, which failed to catalyze any of 50 potential substrates tested. (Further studies on substrate activity are continuing in Hep.G2 and COS-1 cells using the pSVL expression vector.) UDPGT h-1 shows a StuI-polymorphism where both extra 3.2 and 4.3-kb DNA fragments on Southern blot analysis of genomic DNA from individuals are observed. This genotype appeared in 24 of 27 members of the American population (not including Orientals). A screen of DNA from 18 individuals showed that 0/3 Koreans, 1/7 Chinese and 2/8 Japanese contain the extra fragments. Finally, by using UDPGT h-1 as a probe, Owens' group uncovered for the first time a human liver "catechol" estrogen UDP-glucuronosyltransferase clone, UDPGT h-2. The clone is encoded in a 2.1 to 2.2-kb mRNA species. Expression of the insert contained in a pSVL vector in COS-1 cells reveals that a 51 kDa glycosylated protein is synthesized. The preferred substrate activity of the isozyme is as follows: 4-OH estrone > 2-0H estriol > 2-OH estradiol = 6α-OH estradiol > estriol > 4-OH estradiol > 2-OH estrone. It does not glucuronidate the estrogens (β-estradiol or estrone), 16β-estrone, 16α-OH estrone, 16α-OH progesterone, 16α-OH pregnenolone, testosterone,

dihydrotestosterone, androsterone, 16∝-OH testosterone, the catecholamines or some 50 other potential substrates tested. The existence of an isozyme which glucuronidates a comparatively narrow substrate group, including the catechol estrogens, could be crucial in alleviating inhibition by these estrogen derivatives on catecholamine catabolism via their affects on the catechol amine-0-methyltransferase enzyme.

Dr. Samuel Adeniyi-Jones, a member of the Unit on Molecular Biology, has demonstrated that secondary structures affect the function of Alu RNA transcripts in the cell. The alpha fetoprotein beta-1 Alu sequence forms an identical secondary structure with the Alu portion of 7S RNA and can, therefore, bind the same proteins that 7S RNA binds. Differences of a few nucleotides in Alu sequences change the secondary structure of RNAs and since these can have a differential affect on translation, this offers evidence for a role of these RNAs in the regulation of translation. The group has also purified the protein alpha-63 approximately 80%. This protein displays a tissue-specific and developmental pattern of expression in Xenopus. The protein binds primary and processed transcripts of injected repeat sequences in the nucleus and cytoplasm.

The Human Genetics Branch has firmly founded itself in basic research endeavors, which allow the application of current research techniques to the clinical conditions under investigation. Conversely, the human mutations under study provide a basis for the cell biological and developmental studies of the Branch's basic researchers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00131-15 HGB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Human Biochemical Genetics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William A. Gahl	Head	HGB, NICHD
Others:	Isa Bernardini	Chemist	HGB, NICHD
	Megan Adamson	MSF Interinstitute	HGB, NICHD
	Hans Andersson	MSF Interinstitute	HGB, NICHD
	Raili Seppala	Visiting Associate	HGB, NICHD
	Henk Blom	Visiting Fellow	HGB, NICHD
	Thomas Markello	Medical Staff Fellow	HGB, NICHD
	Stephen Kaler	Medical Staff Fellow	HGB, NICHD

COOPERATING UNITS *(if any)*
See Attached

LAB/BRANCH
Human Genetics Branch

SECTION
Section on Human Biochemical Genetics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
6.25	5.25	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1.) Thirty children with cystinosis pre-renal transplant contribute data to a national protocol aimed at determining whether high dose cysteamine/phosphocysteamine is preferable to standard dose therapy. Seven children have objective evidence that cysteamine eyedrops (0.5%) dissolve corneal cystine crystals. Late complications of cystinosis, including myopathy, neurological involvement, and swallowing difficulty, have been described; one cystinotic woman gave birth to a normal boy despite cystine crystals in her placenta. Carnitine therapy continues to be provided for patients with renal Fanconi syndrome.

2.) Central demyelination of various degrees was described in the oculocerebrorenal syndrome of Lowe, and proteinuria was quantitated and characterized. Twenty families have been recruited to this study.

3.) Sialic acid transport across the lysosomal membrane was shown to be defective in infantile free sialic acid storage disease fibroblasts. Sialuria was shown to be caused by a failure of CMP-sialic acid to feedback inhibit UDP N-acetylglucosamine 2-epimerase activity. Free sialic acid was shown to be filtered but not reabsorbed by the human kidney.

4.) A 2-year old boy with hepatic copper storage and aggregates in his fibroblasts helped demonstrate that Indian Childhood Cirrhosis is a genetic disease.

5.) Normal ranges for fetal amino acid and carnitine levels have been reported. Several essential amino acids are concentrated by the fetus.

6.) The lysosomal transport system for tyrosine and other neutral amino acids, discovered in rat FRTL-5 thyroid cells, was shown to be TSH-responsive and identical with a lysosomal transport system for monoiodotyrosine (MIT). The existence of this carrier explains how thyroid cells can salvage thyroglobulin's iodine for reutilization. The use of 125I-DIT as a photoaffinity probe may allow isolation of the gene for the lysosomal MIT/DIT carrier.

7.) Fibroblasts from patients with unknown lysosomal storage diseases are being screened to identify the stored material, with special emphasis upon lipids and carbohydrates.

Cooperating Units:

F. Tietze, Laboratory of Molecular and Cellular Biology, NIDDK
S. Mudd, Laboratory of General and Comparative Biochemistry, NIMH
J. Schneider, University of California at San Diego
J. Thoene, University of Michigan
G. Thomas, Johns Hopkins University
W. Rizzo, Medical College of Virginia
M. Kaiser-Kupfer, Clinical Branch, NEI
H. Levy, Massachusetts General Hospital
J. Schulman, IVF Institute, Fairfax, Virginia
P. Fox, Laboratory of Clinical Investigations and Patient Care, NIDR
V. Hascall, Laboratory of Bone Research, NIDR
M. Dalakas, Laboratory of Medical Neurology, NINCDS
J. Finkelstein, VA Hospital, Washington, D.C.
G. Merriam, Laboratory of Developmental Endocrinology, NICHD
A. Tangerman, Nijmegen, The Netherlands
J. Fink, Laboratory of Developmental and Metabolic Neurology, NINCDS
L. Kohn, Laboratory of Biochemistry and Metabolism, NIDDK
G. Reed, Laboratory of Prevention Research, NICHD
S. O'Regan, Montreal
M. Datiles, Clinical Branch, NEI
T. Kuwabara, Clinical Branch, NEI
Z. Goodman, Laboratory of Hepatic Pathology, AFIP
J. Olson, Johns Hopkins Hospital
L. Plotnick, Johns Hopkins Hospital
R. Reiss, Ohio State University at Columbus
P. Ozand, King Faisal Hospital, Saudi Arabia
A. Yergey, Laboratory of Theoretical and Physical Biology, NICHD
T. Chen, St. Agnes Hospital, Fresno, CA
L. Charnas, Laboratory of Human Genetics, NICHD
G. Harper, Biomedicinska Centrum, Uppsala, Sweden
J. Hopwood, Adelaide Children's Hospital, Australia
K. Horvath, Clinical Center, NIH
C. Oliver, Laboratory of Microbiology and Immunology, NIDR
V. Chaudhry, Clinical Center, NIH
B. Sonies, Clinical Center, NIH
L. Racussan, Johns Hopkins Hospital
G. Ashwell, Laboratory of Biochemistry and Metabolism, NIDDK
G. Barsh, University of California-San Francisco
M. Renlund, Helsinki Children's Hospital, Finland
M. Evans, Wayne State University, Detroit, MI
K. Nicolaides, King's College School of Medicine, London
N. Papadopoulos, Clinical Center, NIH

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00133-12 HGB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Study of Glycogen Storage Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.: James B. Sidbury Adjunct Scientist HGB, NICHD

COOPERATING UNITS *(if any)*

Nutrition Department, CC (P. Brye)

LAB/BRANCH
Human Genetics Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NICHD, NIH Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The study is designed to evaluate the immediate physiological response of patients with glycogen storage disease to different types of raw starches. With time the emphasis has shifted to the potential role of cornstarch therapy in preventing long-term complications.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00137-15 HGB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation and Expression of the UDP-Glucuronosyltransferase Gene Family

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ida S. Owens	Head	HGB, NICHD
Others:	Joseph Ritter	IRTA Fellow	HGB, NICHD
	Yhun Yhong Sheen	Adjunct Scientist	HGB, NICHD
	Serena Farquharson	Biological Aid	HGB, NICHD

COOPERATING UNITS *(if any)*

Brigham and Women's Hospital, Boston, MA (James Crawford)

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Drug Biotransformation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.8	2.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The molecular regulation and genomic organization of the family of genes encoding the UDP-glucuronosyltransferase system are under investigation at the RNA, DNA and protein levels in mice and humans. An undetermined number of transferase isozymes is involved in detoxifying numerous endogenous and exogenous lipophiles through glucuronidation. A mouse liver cDNA, UDPGT m-1, which encodes a transferase expressed in strains AH22 and ZA521 of Saccharomyces cerevisiae, was shown to exhibit a very broad substrate activity. A human cDNA, UDPGT h-1, isolated on the basis of high sequence homology to UDPGT m-1, encodes a 51 kDa microsomal protein when expressed in yeast. This isozyme failed to glucuronidate any of 50 potential substrates tested including bilirubin and is currently under further investigation in the liver Hep-G2 cell and the COS-1 cells. UDPGT h-1 shows a StuI polymorphism by which extra 3.2-and a 4.3-kb DNA fragments on Southern blots appear in the genomic DNA of certain individuals. This genotype is present in 24/27 members of the general American population (not including Oriental members). A screen of DNA from 3 Koreans, 7 Chinese, and 8 Japanese shows that only one Chinese and 2 Japanese contain the extra fragments. In liver, UDPGT h-1 is encoded in 2.3, 3.6, and 5.3-kbase mRNA species attributable to corresponding increments in the lengths of 3' untranslated extensions. The 2.3-kbase species is highly abundant, while the 5.3-kbase is barely detectable. An examination of mRNA from 13 individuals showed that the 2.3-kbase species is missing in one preparation. Finally, we have uncovered for the first time a "catechol" estrogen UDP glucuronosyltransferase clone, UDPGT h-2, from a human liver cDNA library which, when expressed in COS-1 cells, glucuronidates 5 catechol estrogens. It does not glucuronidate the primary estrogens or catechol amines. Certain of these estrogen metabolites are known to inhibit catechol amine metabolism. In this study certain methoxy catechol estrogens (normally formed by the catehol amine-0-methyltransferase) show inhibition of UDPGT h-2 encoded activity. UDPGT h-2 has 95% sequence identity with UDPGT h-1. The molecular mass of the expressed protein is 51 kDa but shifts to 49 kDa when the transfected cells are cultured in the presence of tunicamycin. The activity encoded by UDPGT h-2 may be crucial to the maintenance of normal catehol amine metabolism and action.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00404-07 HGB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cell and Sulfur Metabolism in Fibroblasts of Genetic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jean DeBrohun Butler	Senior Investigator	HGB, NICHD
Others:	Susan L. Owens	Coop Student	HGB, NICHD
	Tammy Hadley	Biology Summer Student	HGB, NICHD

COOPERATING UNITS *(if any)*
Laboratory of Developmental and Metabolic Neurology, NINCDS (P. Pentchev); Environmental Protection Agency (S. Padilla); Laboratory of Cell Biology and Genetics, NIDDK (Mark Levine); Laboratory of Molecular and Cellular Biology, NIDDK (F. Tietze).

LAB/BRANCH
Human Genetics Branch

SECTION
Section on Biochemical Genetics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. Continued studies of mutant mouse which stores cystine in lysosomes as do cystinotic patients; anamolies in cholesterol metabolism uncovered in Niemann-Pick C cells which show lysosomal storage of cholesterol and lack of intracellular cholesterol esterification.

2. Continued studies of cholesterol metabolism and transport in Niemann-Pick C and cystinotic fibroblasts.

3. Characterization of cystinotic cell metallothionein present in a 2-fold excess in cystinotic versus normal fibroblasts.

4. Investigation of alternative ways of depleting cystine levels in cystinotic fibroblasts.

5. Investigation of metabolism of ascorbic acid in normal and cystinotic fibroblasts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00408-06 HGB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathophysiology and Treatment of Human Genetic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Joan C. Marini	Senior Staff Fellow	HGB, NICHD
Others:	Dorothy K. Grange	Medical Staff Fellow	HGB, NICHD
	Mary Beth Lewis	Adjunct Scientist	HGB, NICHD
	Bonnie Orrison	Biologist	HGB, NICHD

COOPERATING UNITS *(if any)*
Laboratory of Bone Research, NID (P.G. Robey); Department of Rehabilitation Medicine, CC (N.L. Gerber); Laboratory of Developmental Endocrinology, NICHD (G. Chrousos).

LAB/BRANCH
Human Genetics Branch

SECTION
Unit on Connective Tissue Disorders

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.6	1.8	1.8

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued our studies to elucidate the molecular basis of heritable disorders of connective tissue and to apply this information to the treatment of the disorders. We have completed the development of the system which we began last year for the detection of point mutations in Type I collagen mRNA by RNase A digestion of mismatches in RNA/RNA hybrids between anti-sense riboprobes and the mRNA of patients. Our anti-sense riboprobe system now covers the entire mRNA for the two genes coding for type I collagen, the major protein of connective tissue. This system allows us to detect and localize mutations in a variety of connective tissue disorders.

We have also developed a complementary methodology for the rapid isolation and sequencing of point mutations detected by RNA/RNA hybrids. This system involves synthesis of first strand cDNA using the patient's mRNA, amplification of the target region on this DNA/RNA hybrid using PCR techniques, and cloning and sequencing of the amplified region.

We have applied this system to the mRNA of patients with Osteogenesis Imperfecta (OI) and have detected mismatches in patients with Types II, III and IV OI. For a patient with Type IV OI we have demonstrated a substitution of serine for glycine in the alpha1(I) chain; for a patient with Type II OI we have demonstrated a substitution of aspartic acid for glycine in the alpha2(I) chain. The pathophysiological implication of these mutations are being investigated.

In clinical protocols, we have continued our investigation of abnormalities of growth-associated hormones in OI and the responsiveness of OI bone to growth stimulation. We have also continued our rehabilitation and bracing protocol for children with moderately severe OI.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00410-04 HGB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Metabolism in Children with Glycogen Storage Disease, Type I

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.: James B. Sidbury Adjunct Scientist HGB, NICHD

COOPERATING UNITS *(if any)*

Laboratory of Theoretical and Physical Biology, NICHD (N. Esteban and A.L. Yergey).

LAB/BRANCH

Human Genetics Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The study is designed to determine the rate of glucose production by the liver in individuals with hepatic glycogenosis. The liver of the individual with glucose-6-phosphatase deficiency does indeed produce glucose.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00412-02 HGB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Molecular Regulation of Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.:	Samuel Adeniyi-Jones	Expert	HGB, NICHD
Others:	Richard Maraia	Medical Staff Fellow	HGB, NICHD
	Susan Adeniyi-Jones	Adjunct Scientist	HGB, NICHD
	Christina Puchalski	Adjunct Scientist	HGB, NICHD

COOPERATING UNITS *(if any)*
Division of Cancer Etiology, NCI (Drs. S. Josephs and M. Klotman); Laboratory of Molecular Biology, NIDDK (Alan Wolfe); University of California (Peter Walter).

LAB/BRANCH
Human Genetics Branch

SECTION
Unit on Molecular Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2	2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Continued work on the biology of expression of Alu-repeat sequences has led to further insight about their hitherto unknown biological functions. We have now been able to elucidate that secondary structures play a role in the function diverse set of Alu RNA transcripts that are made in the cell. Using the alpha fetoprotein beta-1Alu as a model we have shown that it forms the identical secondary structure as the Alu portion of the 7S RNA and can, therefore bind the same proteins that the 7S RNA binds. We had previously shown that the ID sequence transcripts are processed to form small processed RNAs. We can now show that these few nucleotide sequence changes are enough to change the secondary structures of these RNAs and very likely accounts for the differences in interactions and function of these homologous RNAs. Our results also show that these RNAs most likely play a role in the regulation of translation since we can show a differential effect of these RNAs on translation.

The protein alpha-63 is now partially purified approximately 80% and with this we can more thoroughly study the effect of the protein in the transcription of Alu genes. We have now shown that the protein displays both a tissue specific and developmental pattern of expression in Xenopus. Further developmental characterization of the Alu RNAs and the alpha-63 protein is continuing. In addition, the role of the repeats in the regulation of translation during interferon action is being investigated.

Work has continued on the mechanism of transactivation of HIV genes by the TAT gene in the Xenopus oocyte. We can now show regulation at the translational level and have evidence suggesting that this is a major regulatory point for HIV expression in vivo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00909-10 HGB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Fetal Alcohol Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Anil B. Mukherjee	Head	HGB, NICHD
Others:	Sondra W. Levin	Adjunct Scientist	HGB, NICHD
	MoonJohn Kim	Biological Aid	HGB, NICHD

COOPERATING UNITS *(if any)*
Wayne State University, Detroit, MI (M. Evans); University of Mississippi, Jackson, MS (B. Cowan); Vanderbilt University, Nashville, TN (P. Martin).

LAB/BRANCH
Human Genetics Branch

SECTION
Section on Developmental Genetics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.50	0.25	0.25

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [X] (a2) Interviews
- [X] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The investigation on possible genetic predisposing factor(s) for susceptibility to ethanol toxicity is being continued. We have studied a total of five patients with the diagnosis of Fetal Alcohol Syndrome (FAS) under the clinical protocols. Skin fibroblasts from these patients are now being studied for possible biochemical abnormalities in the transketolase enzyme and survival of these cells in thiamine deficient medium *in vitro*. In a related study in animals we have recently discovered that thiamine deficiency during intra uterine development of the fetus in rats alters the response to ethanol in adulthood. Dr. Peter Martin's laboratory at Vanderbilt University has cloned a human transketolase cDNA by antibody screening of a lambda gt 11 human liver library from Clontech. This cDNA will be available for us to probe the Wernicke-Korsakoff fibroblasts which we have established in our laboratory to determine the specific genetic abnormality in transketolase enzyme with high Km for thiamine pyrophosphate which we have reported for this syndrome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00910-10 HGB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biochemistry, Molecular Biology and Physiology of Phospholipase A2 Inhibitory Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Anil B. Mukherjee	Head	HGB, NICHD
Others:	Lucio Miele	Adjunct Scientist	HGB, NICHD
	Antonio Facchiano	Visiting Fellow	HGB, NICHD
	Lalita Murty	Biologist	HGB, NICHD
	Eleonora Cordella-Miele	Visiting Fellow	HGB, NICHD

COOPERATING UNITS *(if any)*
GWU (M. Manyak); U. of Mississippi (B. Cowan); Johns Hopkins (H. Zacur); Johns Hopkins (N. Dubin); Georgetown U. (R. Dhanireddy); Naval Research Lab. (K. Ward) NEI (C. Chan); Israel (D. BenEzra); ATCC (W. Neirman); U. of Albany (C. Baglioni); Walter Reed (S. Levin).

LAB/BRANCH
Human Genetics Branch

SECTION
Section on Developmental Genetics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.0	2.25	0.75

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have obtained very high level expression of recombinant rabbit uteroglobin (UG) in E. coli by means of a T7-promoter based "ATG" vector which we constructed last year. We also found that the recombinant UG produced in bacterial host is solely dimeric, as it is naturally produced in the wet mucosal epithelia of several organs in the rabbit. This recombinant protein is biologically active as a phospholipase A2 (PLA2) inhibitor and inhibits thrombin and collagen-induced platelet aggregation. Site-directed mutagenesis studies are now being carried out with this molecule. The PLA2 inhibitory nonapeptides, derived from uteroglobin and lipocortin, respectively, have a core tetrapeptide (KVLD) in common which is found to be a potent antithrombotic agent in vitro. The mechanism of its antithrombotic action seems to be due to blocking of platelet binding sites for fibrinogen. Thus, when the PLA2 inhibitory peptides are used in conjunction with the tetrapeptide there is an additive effect on the antithrombotic action. Inflammation and blood coagulation are often interrelated and many eicosanoids (prostaglandins, leukotrienes, thromboxane, etc.) play a major role in both of these processes. PLA2 is one of the key regulatory enzymes in the production of arachidonic acid which is required for eicosanoid synthesis. Thus, regulation (activation and inhibition) of this enzyme is vital if we are to understand the mechanisms of many inflammatory diseases. We have recently discovered a novel post-translational modification of this enzyme, mediated by transglutaminase, resulting in activation of PLA2. We found that PLA2 when dimerized by transglutaminase has a 400-fold higher specific activity, as compared to the control. This modification does not change the Km of this enzyme but affects the apparent Vmax which is significantly increased. Further characterization of the kinetic parameters is being pursued. Using a monospecific, polyclonal antibody against rabbit uteroglobin we have screened human prostatic and lung cDNA expression libraries (lambda gt11) and obtained cDNA clones for human uteroglobin-like protein. One of the cloned cDNAs has been analyzed and found to have an insert of approximately 500 bp which is now being sequenced. A cell line has been established by SV40 ts mutant transformation of tracheobronchial epithelia of the rabbit. This cell line secretes uteroglobin upon corticosteroid stimulation. The expression of UG gene in relation to PLA2 and IL-1 genes is now being investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00912-10 HGB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gene Regulation and Cellular Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Janice Y. Chou	Head	HGB, NICHD
Others:	Yu-Jui Yvonne Wan	Senior Staff Fellow	HGB, NICHD
	Takeshi Watanabe	Visiting Fellow	HGB, NICHD
	Juan L. Jimenez-Molina	Visiting Fellow	HGB, NICHD
	Cathie Plouzek	NRC Biotechnology Fellow	HGB, NICHD
	Tsung-Chieh Jackson Wu	Medical Staff Fellow	HGB, NICHD
	John Kasik	IPA	HGB, NICHD

See Attached

COOPERATING UNITS *(if any)* Purdue University, IN (Drs. I. Sun and F.L. Crane); Universitats-Krankenhaus, Federal Republic of Germany (Dr. W. Hopper); Institute of Cell and Tumor Biology, German Cancer Research Center, Federal Republic of Germany (Drs. G. Schutz and S. Ruppert); University of Florida (Dr. D. Chen).

LAB/BRANCH
Human Genetics Branch

SECTION
Cellular Differentiation Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
7.0	6.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We conducted studies on the regulation of gene expression during normal and abnormal differentiation processes. cDNAs encoding the adult formed alpha-fetoprotein (AFP) have been isolated and characterized from cDNA libraries of adult rat liver and cultured rat hepatocytes.

The cDNAs which hybridized with a 1.7-kb transcript in adult liver and cultured hepatocytes share a common 3' sequence (about 1.1-kb) with the 2.2-kb fetal AFP mRNA. However, the 1.7-kb AFP mRNA contains a 240-bp 5' sequence which is absent from the 2.2-kb mRNA and is located in the seventh intron of the fetal rat AFP gene. The two transcripts may be generated by the alternative use of promoters.

Several cDNA and genomic clones encoding human pregnancy-specific beta1-glycoprotein (PSBG), a major glycoprotein produced during pregnancy, have been isolated and characterized. Sequence analysis demonstrated that PSBG is a very polymorphic protein consisting of at least seven species which are encoded by multiple genes. PSBG and its related protein, carcinoembryonic antigen, are members of the immunoglobulin supergene family.

Expression of three liver genes, albumin, tyrosine aminotransferase (TAT) and phosphoenolpyruvate carboxykinase (PEPCK), in the differentiated ts adult rat hepatocytes are regulated by multiple hormones including retinoic acid, cAMP, and glucocorticoids. Expression of all three genes is stimulated by glucocorticoids and cAMP. However, retinoic acid inhibits the expression of albumin and TAT genes induced by glucocorticoids or cAMP, while it enhances expression of the PEPCK gene. These cells provide an excellent model to study hormonal regulation of liver differentiation in vitro.

We demonstrated that in human choriocarcinoma cells, the malignant trophoblasts express the germ cell alkaline phosphatase (AP) gene which is primarily expressed in germ cell tumors. Thus malignant transformation of human placenta suppresses expression of the placental AP gene. We isolated and characterized genomic and cDNA clones encoding the germ-cell AP and found that this gene consists of 11 exons which encode a germ-cell AP mRNA of 2,487 b in length.

Others:	Adam Sartwell	Bio Aid	HGB, NICHD
	Chi-Jiunn Pan	Adjunct Technician	HGB, NICHD
	Ke-jian Lei	Courtesy Investigator	HGB, NICHD

LABORATORY OF COMPARATIVE ETHOLOGY (LCE)

Z01 HD 00054-15	Structural and Behavioral Analysis of Vocal Communication in Squirrel Monkeys David Symmes, Ph.D.
Z01 HD 00062-13	Physiological Control of Vocal Production in Squirrel Monkeys John D. Newman, Ph.D.
Z01 HD 00702-09	Genetics of Primate Vocal Behavior John D. Newman, Ph.D.
Z01 HD 01106-06	Developmental Continuity of Individual Differences in Rhesus Monkey Reactivity Stephen J. Suomi, Ph.D.
ZO1 HD 01107-06	Adaptation of Laboratory Reared Monkeys to Field Environments Stephen J. Suomi, Ph.D.
Z01 HD 01108-05	Comparative Studies of Play Behavior Maxeen Biben, Ph.D.
Z01 HD 01110-02	Intuitive Parenting of Infants in Comparative Perspectives (Inactive)
Z01 HD 01111-04	Factors Affecting Nurturant Behavior Toward Infants Frank A. Pedersen, Ph.D.
Z01 HD 01112-03	Effects of Home- and Out-of-Home Care on Child Development Michael E. Lamb, Ph.D.
Z01 HD 01113-03	Antecedents, Correlates, and Consequences of Adolescent Pregnancy and Parenthood Michael E. Lamb, Ph.D.
ZO1 HD 01114-02	Individual Differences in Physical and Affective Functioning in Infancy Michael E. Lamb, Ph.D.
ZO1 HD 01115-02	Effects of Domestic Violence on Children's Development Michael E. Lamb, Ph.D.
ZO1 HD 01116-02	Pattern of Childrearing Across Cultures and Ecologies Michael E. Lamb, Ph.D.
ZO1 HD 01117-02	The Hospitalization Experience: Children's Coping with the Stress of Surgery Marc H. Bornstein, Ph.D.

ZO1 HD 01118-02	Latent Behavioral Effects of Diverse Forms of Caretaking in the First Year of Life Marc H. Bornstein, Ph.D.
ZO1 HD 01119-02	Specificity of Mother-Infant Interaction Marc H. Bornstein, Ph.D.
ZO1 HD 01120-02	Observations of Parenting and Infant Activity in Different Societies Marc H. Bornstein, Ph.D.
ZO1 HD 01121-02	Parental Activities and Children's Language and Play Marc H. Bornstein, Ph.D.
ZO1 HD 01122-02	Assessment of Children's Mental and Social Abilities Marc H. Bornstein, Ph.D.

Laboratory of Comparative Ethology

The Laboratory of Comparative Ethology (LCE) carries out a program of research investigating behavioral and biological development in humans and in nonhuman primates. The influences of both genetic and environmental factors -- and their multiple interactions -- are explored in a comparative approach in order to characterize the origins, ontogeny, and evolution of various behavioral phenotypes. Experimental results in nonhuman primates are correlated with findings from longitudinal studies of human infants and their families, as well as with data obtained by neuroscience techniques. Longitudinal designs are employed to address issues of ontogenic continuity vs. change, and in many of the investigations a variety of both behavioral and biological measures reflecting multiple levels of analysis are collected concomitantly. A major emphasis is placed on characterizing and understanding normative patterns of biobehavioral development so that deviant patterns can be readily identified and their consequences evaluated with respect to established norms.

The LCE consist of four sections. The Comparative Behavioral Genetics Section, headed by Dr. Suomi, investigates various processes underlying biological and behavioral development in selected nonhuman primate species by focusing on interactions between genetic and environmental factors that affect the course of an individual's ontogeny. Within the Section, the Unit on Neuroethology, headed by Dr. Newman, uses neuroscience techniques to study brain mechanisms involved in the production of various types of primate vocalizations by squirrel monkeys and to examine subtle acoustical differences in characteristic calls among several primate species. The Brain, Behavior, and Communication Section, headed by Dr. Symmes, studies the production and utilization of vocal signals by group-living squirrel monkeys in terms of both the acoustical properties of the signals and their information content for group members. Parallel acoustical analyses are conducted on selected vocal patterns of other primate species, including humans. The Child and Family Research Section, headed by Dr. Bornstein, examines perceptual, cognitive, and dispositional development in human infants and children, with special emphasis on studying the relationships among early attentional processes, social stimulation from caregivers, and subsequent cognitive capabilities. The Section on Social and Emotional Development, headed by Dr. Lamb, studies the effects of different types of caregiving practices on infant and toddler social and emotional development and cognitive competence. Special attention is given to longitudinal approaches that involve cross-cultural comparisons and those examining nonnormative samples of both parents and infants. Additional investigations of parent-infant relationships are carried out in the Unit on Parent and Infant Studies headed by Dr. Pedersen.

Research in the Comparative Behavioral Genetics Section (CBGS) during FY89 continued its primary focus on characterizing individual differences among rhesus monkeys in their biobehavioral responses to environmental novelty and/or challenge, determining how genetic and environmental factors interact to shape such individual differences, and assessing the long-term developmental consequences of these different response styles. In one major study cross-fostering techniques were utilized to evaluate the effects of different types of maternal care on rhesus monkey infants selectively bred to be unusually reactive to novelty or challenge, compared with infants selected to display more normal biobehavioral response styles. Thus experienced multiparous

monkey females who differed systematically in their characteristic maternal "style" raised infants who differed in their genetic risk for extreme reactions to environmental novelty and challenge. Infants whose pedigree put them at either high or low risk for reactivity were cross-fostered within the first 4 days of life to these multiparous females, who either had consistently demonstrated a highly nurturant style of care toward previous offspring or who had demonstrated species-normative levels of support, punishment, and rejection.

Analyses completed in FY89 of 3 independent cohorts revealed that high reactive infants differed systematically from control infants in their neonatal test profiles but displayed normal patterns of behavioral development during their 6 months with foster mothers. In fact, high-reactive infants cross-fostered with nurturant females actually exhibited accelerated developmental curves relative not only to high-reactive infants reared by control foster mothers but also to control infants reared by either kind of foster mother. However, when challenged by brief maternal separation at 6 months of age, high-reactive-pedigree infants displayed extreme behavioral and adrenocortical reactions regardless of their respective foster mothers. By contrast, control infants exhibited much milder reactions to challenge, but only if they had been reared by nurturant foster mothers. These patterns were reversed immediately after reunion. Long-term follow-up of the first cohort of these cross-fostered monkeys, now entering puberty, has revealed that the unexpected social advantage apparently conveyed to high-reactive youngsters by nurturant foster mothers clearly carries over to subsequent situations in which social support networks are available, but not to other situations. These findings provide a clear and important demonstration that genetic predispositions can be modified substantially by specific environmental events during development and that the nature of the modification is dependent both on previous experiences and the nature of the current environmental situation.

Data from a second ongoing longitudinal study of individual differences in rhesus monkey biobehavioral response to challenge has provided compelling evidence that such individual differences also occur among wild-living monkeys and are likely to be of substantial biological significance. In this study young rhesus monkey males living in a free-ranging natural troop on Cayo Santiago, PR were studied as they passed through puberty, a period during which males in the wild typically emigrate from their natal troop and attempt to join new social groups. Measures of cardiovascular, adrenocortical, and behavioral response to environmental challenge were obtained from these pubertal males during each of two brief periods of capture and physical restraint for routine annual veterinary examinations. In addition, daily behavioral observations were carried out on each subject before, during, and following emigration from its natal troop. Thus, it was possible to characterize each of these young males in terms of relative biobehavioral reactivity to a standardized challenge and then monitor their activities in a wild setting during a crucial period of developmental transition.

Results to date have revealed substantial individual differences among these wild males in their biobehavioral response to the capture-and-restraint challenge, with values that mirror those seen in captive pubescent rhesus monkey males -- and with remarkable year-to-year stability of these individual differences. Strikingly, high-reactive males (i.e., those with higher and more stable heartrates, lower vagal tones, and higher levels of plasma cortisol) tended to leave their natal troop later chronologically than low-reactive males. Moreover, when they did finally leave their natal troop, these high reactive males usually failed to join all-male "gangs," as is typical for most adolescent males emigrating in feral settings, but instead tried to go directly into a new troop, albeit limiting their interactions largely to low-ranking members on the periphery of the new troop. Ongoing observations will permit determination of the relative success

of the different emigration "strategies" displayed by these high and low reactive males. The discovery of different male dispersal strategies associated with differential biobehavioral reactivity to brief challenge is especially striking in that other, more "traditional," measures of these males' chronological, physical, and hormonal maturity, as well as social status in their natal troop, failed to predict these different strategies. Thus, behavioral and biological assays developed to characterize relative reactivity among laboratory-reared captive rhesus monkeys not only can be used to demonstrate similar patterns of interindividual variation in wild populations, but they also turn out to be the best current predictors of individual patterns of dispersal among pubertal males growing up in feral troops, a topic of intense current interest among behavioral ecologists and evolutionary biologists.

Given these and other findings documenting major sex differences in rhesus monkey life course clearly emerging around puberty, the CBGS during FY89 directed increased resources toward characterizing species-normative patterns of biobehavioral continuity and change throughout the transition through puberty. Data collected in long-term longitudinal studies documented systematic changes in several different biobehavioral systems that appeared during adolescence. First, results from 3 independent longitudinal samples of subjects growing up in different settings demonstrated an apparent suppression of adrenocortical response to environmental challenge in male, but not female, monkeys as they entered adolescence. Pubertal males born and raised in a wild rhesus monkey troop, as well as laboratory born males either reared by their biological mothers in age-graded social groups or hand-reared in a nursery and subsequently put into mixed-sex peer groups, all showed significant decreases from juvenile levels of plasma cortisol following challenge, whereas most females either exhibited stable or developmentally increasing cortisol values. In a similar vein, rhesus monkey males had significantly greater drops in CSF levels of the serotonin metabolite 5-HIAA as they approached adolescence than did females, a pattern that closely tracked developmental increases and emerging sex differences in the absolute frequency and relative intensity of aggressive behavior.

These characterizations of normative patterns of biobehavioral functioning during a period of major developmental transition have provided two new insights. First, the most dramatic behavioral, adrenocortical, and monoamine developmental changes in rhesus monkeys seem to precede the most obvious changes in physical features and sex steroid levels, in some cases by as much as a year's time. This is not true for other biobehavioral measures generally sensitive to environmental challenge, such as levels of plasma ACTH or CSF MHPG. Second, there is remarkable stability of individual differences on each of these disparate measures of response to challenge prior to and generally throughout the pubertal period, despite the dramatic changes in absolute levels of these measures during that same period. Such findings suggest that individuals who will exhibit extreme reactions to challenge during the relatively turbulent times of adolescence can be identified well before the onset of obvious physical pubertal change.

A quite different set of studies investigating patterns of response in the face of environmental novelty focused on biobehavioral consequences of various efforts to "enrich" the living environments of captive primates, a research topic now relevant in light of recent amendments of the Animal Welfare Act concerning the maintenance of nonhuman primates in captivity. Four different forms of "environmental enrichment" were systematically introduced to monkeys living in otherwise stable physical and social environments. In each case, these fundamentally different enrichments -- a fleece-covered "grooming board" for individually housed adults, a 1/4-acre enclosure periodically available to members of family groups, a provision of cover designed to

promote foraging behavior among social group members, and systematic distribution of preferred food items within "foraging bins" -- clearly altered behavioral profiles of most subjects in directions consistent with current notions regarding well-being in captive nonhuman primates. These findings have already led to specific changes in some SOPs for animal care in the LCE primate colony.

Finally, during FY89 several investigations continued to characterize individual differences in reflex development, activity state patterns, and temperament during the neonatal period among nursery reared rhesus monkey infants and selected comparison species. A major goal of these research efforts centers around the use of neonatal measures to predict various patterns of individual differences during later developmental periods. In one study, differences among rhesus monkey infants in fussiness during the first month were associated with the relative power of environmental enrichment to enhance cognitive and social-emotional development--fussy (high reactive) infants benefited less from two types of extra environmental stimulation than did control rhesus monkey infants. A second study, in collaboration with the New England Regional Primate Center, discovered that different nursery procedures were associated with differences in neonatal test scores, in some cases exceeding differences between infants of different macaque species reared in the same nursery environment. The significance of these latter findings lies in the demonstration that neonatal temperamental characteristics are apparently subject to environmental modification within the first few days and weeks of life, at least in macaque monkeys. Other collaborative studies utilized standardized neonatal assessments to evaluate the efficacy of prenatal intervention for hydrocephalia in rhesus monkey infants, to predict cognitive and social-emotional functioning in young chimpanzees, and to establish normative developmental standards for captive Cebus apella infants.

A major topic of research in the Section on Social and Emotional Development (SSED) during FY89 also focused on characterizing individual differences in early reflex development, patterns of arousal and activity, and temperament in early infancy, but in this case among selected samples of young human children. Just as in the previously described rhesus monkey studies, the overall objectives of these very early assessments of human infants have been both to understand the interrelationships among different behavioral and physiological systems at this stage of development and to determine the relative efficacy of these various measures in predicting patterns of individual differences later in infancy and into childhood.

In one major study, 5-month-old infants selected on the basis of parental reports of extreme behavioral and physiological arousal during their initial months (e.g., frequent constipation or colic, sleeping difficulties, respiratory or skin allergies, strongly adverse behavioral reactions to environmental changes, and extreme irritability) were compared with same-age control infants in terms of a variety of psychophysiological measures (heartrate, heartrate variability, and vagal tone) and levels of saliva cortisol obtained in a controlled laboratory setting, temperament (as assessed by a standardized maternal questionnaire), and daily activity patterns (as recorded by mothers in diary fashion over a 1-week period in the home). The infants were then followed longitudinally in standardized laboratory sessions and home visits at 7, 10, and 13 months of age in order to obtain additional age-appropriate measures of cardiovascular and adrenocortical activity, temperament and emotional responsivity, and interaction patterns and attachment relationships with their mothers. Preliminary analyses of data from the initial phase of the study revealed that infants who were described as extremely irritable by 4 months of age scored higher than nonirritable babies on the fussy and unadaptable subscores of the temperament index when they were 5 months old.

Irritable babies were more likely to have extremely high or low vagal tone, and extremely high or low heart rate, than were nonirritable babies. Additionally, infants with extremely high or low vagal tone or heart rate appeared more fussy and unadaptable on the relevant temperament subscales. Finally, children characterized by symptoms of irritability differed significantly from children with no such symptoms on a derived measure representing the absolute distance from the grand mean of the vagal tone measure. Only scores on this derived variable accounted for a significant amount of variance between the two groups (irritable vs. not irritable) in a step-wise discriminant function analysis. These findings are significant in that they demonstrate convergence of both behavioral and physiological indices of extreme patterns of fussiness and arousal by 5 months of age, considerably earlier chronologically than has been reported for human infants in previous studies. Comparisons of these 5-month data with the results of the subsequent laboratory and home visits are currently underway.

A second area of interest common to SSEC researchers and previously described nonhuman primate work focuses on study of developmental continuity and change through puberty and adolescence and into early adulthood. The goals of these efforts include not only identifying normative patterns of social, emotional, cognitive, and physiological functioning in adolescence but also identifying aberrant or extreme patterns and determining the long-term consequences. One aspect of this work involves use of large scale national surveys of adolescent youth and their children, especially as they relate to teenage pregnancy and parenthood. Analyses of survey data completed during the past year indicated that teenage mothers were much more likely to have exhibited a greater array of antisocial, educational, and substance abuse problems prior to pregnancy than were adolescent females who did not become pregnant but were otherwise matched for age, race, and socioeconomic background. This pattern of early problems was very similar to that previously reported for adolescent males who became fathers during their teens. Moreover, in appropriately matched comparisons with adolescent females who subsequently either had their first child between 19 and 21 years of age or who were still childless at 21, teenage mothers were more like than either young adult mothers or non-mothers to have records of school suspension, truancy, running away, smoking marijuana, and fighting. There were variations in the results by race and geographical area: Blacks reported fewer problem behaviors than did Whites, and urban women engaged in fewer problems behavior than did rural women. Young urban women who engaged in three or more problem behaviors were more likely than women who reported no problem behavior to subsequently have a child prior to age 19. These findings contradict the notion that adolescent parenthood is a random event, with some proportion of sexually-active youngsters unlucky enough to get caught. In fact, the findings suggest that adolescent parenthood is but one symptom of psychosocially-troubled youths, illustrating the multi-problem nature of adolescent parenthood and the need for a clear and comprehensive understanding of the context of adolescent pregnancy.

In a related study, national survey data were utilized to evaluate long-term consequences of adolescent parenthood and marriage. A striking finding was that adolescent marriage was associated in both men and women with deficits in marital stability, income, educational attainment, and occupational prestige up to 40 years after the marriage. Clearly, adolescent marriage can have adverse consequences that endure throughout men's and women's working lives. This finding was especially noteworthy because it has often been assumed that men are spared adverse effects because they do not have to endure the pregnancy and traditionally play a minor role in childcare.

A third major research focus of the SSED in FY89 involved continued longitudinal study of the effects of different types of infant care arrangements (home care, family daycare, or center-based daycare) on social, personality, and intellectual development in a large, carefully selected sample of toddlers. The different care arrangements began when the children were 16 months old, and annual assessments of their social, emotional, and cognitive patterns and capabilities have continued to the present (these children are now beginning their first grade of school). Results of data analyzed to date have consistently demonstrated that the type of care arrangement by itself was not associated with major differences in child outcome measures at any time during the toddler and young childhood years. In contrast, beginning in the second year assessments, quality of home care (itself correlated with family social class), infant temperamental difficulty, and perceived social support were the most influential factors. Two years after enrollment, quality of home care continued to have the most consistent impact on personality maturity and emergent social skills with peers and adults. The quality of alternative care now also had an effect, albeit one that was inconsistent and more modest than that associated with quality of home care. The impact of the quality of alternative care became more dramatic when the indices of quality were expanded to include observational measures of the children, careproviders, and settings. Later analyses examining children's compliance with maternal requests in a task-like setting revealed that the quality of home care was the best predictor of both the mothers' and children's behavior. The same pattern of relative influence was also found for measures of cognitive capabilities, with quality of home care accounting for the largest share of the variance at each age tested. The quality and extent of nonparental care had smaller but significant impacts as well. Ongoing research is designed to further explore the determinants of individual differences in the mothers' behavior -- a key component of the quality of home care.

In view of these and other findings demonstrating the importance of various aspects of home care during infancy, toddlerhood, and early childhood, the SSED has initiated a series of cross-cultural studies designed to characterize various aspects of the home environment across different social and physical ecologies, especially in terms of parental beliefs and values, parenting programs, and how differences in these domains affect children's development. In the first study, SSED staff collected interview data about perceptions, values, expectations, responsibilities, and practices of West African parents. These data are being analyzed and will be interpreted in the context of information about variation in developmental niches. In a second study completed during FY89, SSED staff explored the effects of agreement between Swedish mothers and fathers regarding socialization values. Agreement between parents was less clearly associated with developmental outcomes in Sweden than in the USA. In a third study, SSED staff are attempting to assess specific maternal and child attributions about one another in order to identify the extent to which attributions or expectations shape the way grade school-age children interact with their parents. Finally, SSED staff are exploring the validity of Strange Situation assessments of infant-mother attachment security in Japanese dyads.

Cross-cultural research designs also provided the basis for several major projects in the Child and Family Research Section (CFRS) during FY89. In these studies the primary focus has been to specify the nature of infant-caretaker relations among infants living in different normal urban environments and infants living in different atypical environments including, traditional kibbutzim in Israel, middle-class homes in urban settings in Japan, France, and the U.S.A., and lower-class homes in Argentina and the U.S.A. The goals of these ongoing longitudinal studies are to identify and describe similarities and differences in common activities of mothers and infants in different cultures and to contrast the nature of basic early mother-infant interactions

in different societies. Further, information is being obtained on assessing infant information-processing capabilities and on infant temperament.

During the past year data collection and analyses comparing the Japanese and U.S. mother-infant cohorts when the infants were 5, 13, and 20 months of age were completed. Different patterns of mother-infant interaction and different communicative styles of maternal speech were found between the two countries. The results also identified cultural-specific maternal behaviors and speech in each country. Analyses of maternal speech and mother and infant behaviors at 13 and 20 months have thus far uncovered activity and interaction patterns which are distinctive to these two disparate cultures as well as patterns which are similar between the two cultures and which may point to processes universal in early development. Additionally, habituation (to mother's face and to a single familiar object) was studied from videotaped segments in each sample. These observations also indicate that habituation is a commonplace response of infants to faces and to objects naturally encountered in everyday settings. Moreover, the characteristics of habituation appear to be similar to those measured in the laboratory, and samples of Japanese and American infants appear to habituate similarly. Data collection for the other cross-cultural samples continued during FY89.

Detailed longitudinal study of patterns of mother-infant interaction provided the basis for several other major research projects in the CFRS this past year. A common objective of these studies has been to investigate both short- and long-term influences of caregiver behavior on cognitive development in infancy and early childhood, especially among individuals who exhibit different patterns of habituation during their initial months of life. The rationale for this emphasis stems from the fact that before children are old enough to enter formal social learning situations, nearly all of their experiences stem direction from interactions they have with their primary caretaker. In these studies caretaker-child interactions can be conceptualized as falling into four distinct categories: nurturant, material, social, and didactic. Mother-infant interactions are typically videotaped for subsequent microanalysis in both home and laboratory settings. In most of these studies the interaction sessions are conducted when the infants are 2, 5, 13, and 20 months of age.

During FY89 data analyses were completed on a large normative sample of infants and their caretakers. At 2 and 5 months of age, mothers' activities were somewhat stable, but largely independent at the two ages (i.e., some were characterized by continuity whereas others were discontinuous). Infants' activities were generally unstable, also independent, and generally increasing over time. Mothers' encouragement of infants' attention and infant habituation were then examined at 5 months in relation to toddlers' play, language comprehension, and representational competence at 13 months. Early maternal encouragement explained unique variance in language comprehension and representational competence after partialling habituation, and habituation predicted play, language comprehension, and representational competence after the influences of both early and later stimulation were partialled. These findings indicate that maternal stimulation predicts toddler cognitive ability over and above infant habituation, and also that links between habituation and cognition are not solely mediated by maternal stimulation.

In the same study, relations among toddlers' language production, language comprehension, play competence, and attention span at 13 months were examined with and without mothers' didactic encouragement partialled. Language production and flexible language comprehension systematically covaried, and play competence systematically covaried with flexible language comprehension and with attention span. These relations maintained even when concurrent maternal stimulation was partialled.

In addition, mothers' contingent responsiveness to their 5-month-olds predicted both representational competence (play and language comprehension) and exploratory competence, even after infant habituation and early and later maternal noncontingent stimulation were partialled. This finding suggests that contingent responsiveness in infancy uniquely predicts competencies at the start of the second year.

In assessment of mother and toddler interaction, mothers' demonstrations and elicitations of nonsymbolic and symbolic play were examined in relation to toddlers' nonsymbolic and symbolic play between 13 and 20 months. The relative sophistication of mothers' and toddlers' play closely paralleled one another at both ages. Mothers were highly stable on all activities, whereas only nonsymbolic play in toddlers was stable. At both 13 and 20 months, individual differences among mothers and among toddlers were associated with one another in highly specific ways. Mothers' symbolic play related to children's symbolic play, and their nonsymbolic play related to children's nonsymbolic play. However, neither mothers' play nor toddlers' early play demonstrated any predictive influence on the later activities of the other.

The potential consequences of early disruptions in infant-caregiver interactions were seen in results of a retrospective study in which childrens' social, emotional, and cognitive competencies were assessed at 4 years of age and then examined with respect to the caregiving status of the mothers during the first few months of life. Preliminary analyses of data from the first 60 subjects reveal significant differences among children at 4 years of age who experienced varying modes of care in the first 6 months of life. For example, children cared for by their own mothers in the first 6 months of life had higher Adjustment scale scores than agemates who experienced increased amounts of nonmaternal care in the company of other children in the first 6 months of life. Higher Adjustment scores indicated better performance on intellectual (cognitive and verbal) assessments and lower number of disturbed behaviors, as indicated on the Preschool Behavior Questionnaire (PBQ), including hostile/aggressive, anxious, and hyperactive/distractible behaviors. In addition, children who, in the first 6 months of life, had less stable nonmaternal care (i.e., multiple caregivers and locations of care which translated into higher numbers of changes in their care histories) scored significantly lower on the cognitive index and the perceptual/performance scale of the McCarthy Scales of Children's Abilities. These findings held even when concurrent caretaking circumstances at 4 years and maternal education level were taken into account. These findings have important policy implications regarding the advisability of out-of-home care for infants prior to 6 months of age, and they have provided the motivation to carry out a large-scale prospective study of infants whose caretaking experiences vary systematically in their first few months of life.

A final longitudinal study investigating mother-infant interactions focused on specific aspects of emerging language capabilities and play activities at 13 and 20 months. Analyses of interaction patterns at 13 months revealed that maternal didactic interactions, but not maternal social interactions, related independently to toddlers' language, not play, skills. Conditional associations between domains significantly augmented explained variance in both toddler language and play. For example, social interaction was positively associated with language only in dyads where mothers, rather than toddlers, controlled object-oriented exchanges. Neither social nor didactic interactions were independently associated with toddler pretense play, but their combination was. Conditional relations between interactions and toddler skills suggest the importance of maternal modulation of social and didactic exchanges depending on toddler attention and initiative. Longitudinal data were analyzed in order to uncover predictive relations between mother-toddler interaction at 13 months, toddler

competence at 13 months, and toddler competence at 20 months. The results showed strong continuities between toddler language performance at 13 and 20 months, but little correspondence between 13- and 20-month play performance.

Further examination of 13-month toddler data yielded an interesting and unexpected result: Despite the fact that all toddlers were evaluated within several weeks of their 13-month birthdays, subtle variation in toddler age was related to language comprehension assessed by two independent measures -- a standardized instrument and a detailed maternal interview. The association of toddler age with language comprehension at such an early age, when age variation was quite minimal, is noteworthy. This finding has potential technical relevance to other researchers in the area of language development, and the data form the basis for a manuscript currently in preparation.

Caregiver-infant interactions and patterns of social play also provided the basis for major research efforts in the Brain, Behavior, and Communications Section (BBCS) this past year. Longitudinal study of the emergence of vocal communication in socially reared squirrel monkeys revealed interesting qualitative changes in the nature and apparent target of vocal utterances displayed by infants during their first few months of life, especially when the mothers and infants were housed in small social groups instead of in isolated dyad cages. Isolated mothers averaged less than 1 call per hour to their infants in the first month of life, while youngsters are still on the mothers' backs almost continuously. In contrast, a mean of 81 calls per hour was directed to infants housed in a small group during month 1. 93% of these were from aunts, including those carrying their own babies; only 7% of these calls were from an infant's own mother. This ratio changed abruptly when infants began to leave their mothers' backs in month 2 and 3: aunts' rates fell precipitously while mothers increased their rates to keep in contact with wandering infants. Almost all of the vocalizations directed to infants in the first 2 months were "caregiver calls," a type which shows many similarities to "motherese" in humans. Infants responded, either vocally or with vocalizations plus gestures, to 15-20% of caregiver calls in the first month, increasing to 30-50% in month 2. Particularly in the first month, most infant vocalizing was responsive, not spontaneous, under these low stress conditions. About 75% of infant utterances were made in response to caregiver calls directed to them at close range, usually by aunts. Infants (1-4 weeks old) in small groups emitted an average of 19 calls per hour, compared to 7 per hour for infants housed along with their mothers.

Continued study of vocalizations emitted by juvenile squirrel monkeys during bouts of active play revealed that the primary function of "play" vocalization was to alert adult group members to be more vigilant when the young are absorbed in play. Play has been shown to be a risky activity in other primates, exposing the vulnerable young to predation. However, the protection afforded by adults monitoring such activity allows youngsters to play with abandon and in large numbers and compensates for what would otherwise be a maladaptive activity where animals crash through the trees, vocalizing loudly and oblivious to predators. This finding is further evidence for both the importance of play and the degree and variety of indirect parental care in this species.

Finally, during FY89 the BBCS computer system was used to analyze vocalizations collected from juvenile rhesus monkeys briefly separated from their natural troop (in Puerto Rico) during routine veterinary examinations in a collaboration with the CBGS, the Unit on Neuroethology, and the Caribbean Primate Research Center. A major finding was that the peak frequency and duration of "coo" calls changes systematically throughout the 24-hour period of separation. Moreover, the relative values of several vocal parameters of these calls also were systematically related to levels of plasma

cortisol and ACTH, as well as lymphocyte counts in blood samples obtained during the separation.

Another study carried out in the Unit on Neuroethology also focused on the vocal patterns of wild-living monkeys, in this case a troop of free-ranging squirrel monkey in Costa Rica. The objectives of this study were to characterize the relationship between troop activity, spatial distance between troop members, and vocal patterns. Twenty-one adult female squirrel monkeys were marked with Nyanzol dye, facilitating individual identification. After a 3-week period of habituation, vocalizations were recorded onto a tape recorder along with ongoing activity of the vocalizer, distance to the nearest adult female, and troop activity. Subsequent analysis in the laboratory consisted of producing sound spectrograms for each vocalization of suitable quality, classifying the vocalization as to subtype (peep, twitter, smooth chuck, bent mast chuck), and assigning a unique code according to its place in a recording bout. Each call was then associated with the concomitant behavior of the vocalizer and distance to the nearest adult female. Statistical analysis revealed that distance of the vocalizer to the nearest adult female was a highly significant factor affecting rate of calling. For "peeps," the category containing the species-specific isolation call, there was a significantly greater incidence of these sounds at distances greater than 1 meter from the nearest adult female, whereas other vocal subtypes showed no significant correlation with production rate and distance from the nearest neighboring female. No significant association was found between behavior of the vocalizer (foraging vs. nonforaging or stationary vs. traveling) and type of vocalization.

Another study completed in FY89 focused on the role of catecholamines in mediating isolation call production in adult male squirrel monkeys. In this study, conducted in collaboration with the Laboratory of Clinical Science, NIMH, peripherally administered drugs known to selectively inhibit either the A or B form of MAO were tested over a 5-fold dose range on production of the species-typical isolation call of squirrel monkeys ("isolation peep," IP). In contrast to 2 drugs with MAO-B inhibitory activity (milacemide and l-deprenyl), the MAO-A selective drug clorgyline produced a dose-related decrease in IP production, but failed to completely suppress IP production even at the highest dose employed (5 mg/kg), and likewise failed to produce a significant decrease in locomotory activity. These results suggest that the catecholamine substrates of endogenous MAO-A enzyme (e.g., noradrenalin and serotonin) are likely to play a more central role in regulating IP production relative to other aspects of behavioral arousal such as locomotion. The substrates of endogenous MAO-B, on the other hand, appear to be less directly involved in regulating vocal behavior.

An ongoing project showing considerable progress this year involved the longitudinal assessment of the vocalizations of rhesus macaques subjected prenatally to a cerebro-ventricular shunt to correct experimentally induced hydrocephalus, done in collaboration with Drs. Suomi and Michejda. Additional experimental and age-matched control subjects were studied, with a continued affirmation of the differences reported last year (experimentals producing more noisy and variable calls during brief social separation). More detailed analysis this year has revealed that there is a significant gender difference in the incidence of noisy vs tonal separation calls in the control animals, with females producing a greater proportion of noisy calls. By separating male and female experimentals prior to statistical analysis, it was shown that female experimentals are not statistically different from their control counterparts, whereas male experimentals produce significant less tonal and significantly more noisy calls ("leap-coos") than control males. Increased noise and variability has been shown to be a valid measure of increased distress or agitation in human infants, and may be an indication in the present study that females (and the experimental males) are more

agitated when socially separated than are control males. NMR scans performed on all of the experimental animals failed to reveal any abnormalities in size or shape of the cerebral ventricles. However, a potential neural substrate for these vocal differences has been identified, in a collaborative study with Dr. Bachevalier of the Laboratory of Neuropsychology, NIMH, in this case involving the longitudinal assessment of vocalizations in rhesus macaques receiving bilateral ablation of the inferotemporal cortex. Analysis of recordings made over the first month following surgery revealed that the vocal behavior of the experimentals is very similar to that of the corrected hydrocephalus animals, with both male and female IT subjects showing reduced tonal and increased noisy calls during brief periods of social separation. As with the corrected hydrocephalus subjects, it is the males that showed a significantly different ratio of tonal and noisy calls in comparison to age-matched controls of the same sex.

Finally, the Unit on Neuroethology conducted systematic analyses of human infant vocalizations as part of a large, longitudinal project being carried out in the Unit on Parent and Infant Studies. This project, for which data collection was completed in FY89, investigated emotional factors ascertainable in parents during the pregnancy period, reactivity to infant cries that have varying degrees of aversiveness, and the unique individuality of the infant as factors that collectively influence the course of the parent-infant relationship in the first year of life. First-time expectant mothers and their spouses were studied prior to and immediately following the infants' birth, and parents and infants were followed at 3, 9, and 12 months. Nonpregnant women were also studied with a subset of the procedures at times corresponding to the prenatal and 3 month periods. The study employed multiple levels of measurement, including observational, self-report, and physiological procedures. Preliminary analyses, particularly for the prenatal and 3 month periods, have yielded several important findings.

First, physiological reactivity, as assessed by changes in heartrate, to the mildly stressful stimuli of recorded infant cries was attenuated among pregnant compared to non-pregnant women, even though both groups were generally similar in their subjective ratings of cries with differing degrees of aversiveness. On subjective ratings there were highly significant discriminations of the cries into two groups, one very aversive and one relatively nonaversive. Pregnancy status had no effect. On physiological measures of reactivity (heart rate change to the cry stimuli), the aversive cries elicited a pattern of response classically identified as more stressful than did the nonaversive cries. Independently of cry type, pregnant women showed a less reactive pattern after the first few seconds than was evident for the nonpregnant women. Vagal tone, a measure of heart rate variability that provides an independent measure of stress reactivity, was found to be in the lower range for a greater proportion of pregnant women than for the nonpregnant group. When the combined group was classified on high, medium, and low vagal tone, the low group (which contained primarily pregnant women -- 16 vs. 5), had a clear-cut dampened reactivity pattern. This suggests that pregnancy normally results in some attenuation of reactivity of the autonomic nervous system. Pregnant women whose vagal tone was not in the lower range also scored significantly higher in their reports of anxiety symptoms, suggesting that dampened reactivity may have a protective influence during fetal development.

A second set of findings relates to infant individuality. In connection with the neonatal assessments, brief recordings of the infant's cry were obtained. Cry samples were also obtained at age 3 months. These samples (N = 36) were analyzed for 8 acoustic characteristics. Four acoustic parameters were significantly correlated between the neonatal and 3-month period, indicating stable individual differences. Three of these dimensions were significantly related to maternal ratings of infant

temperament. For example, a high fundamental frequency in the cry was related to parental ratings of the infant being unadaptable, unpredictable, and having a "difficult" temperament. These results extend into the normal range some of the conclusions that have previously been drawn with clinically significant groups of infants.

Other results bearing on infant individuality were found in relation to the measure of heart rate variability, vagal tone. Infants whose baseline vagal tone at age 3 months was in the higher range were found in a laboratory procedure to have a different visual fixation pattern that was consonant with more rapid processing of visual stimuli. They also were rated in the laboratory procedure as more soothable, and fewer infants in the high vagal tone group required soothing interventions. Moreover, ratings of negative affect in these infants were significantly lower. As in the findings involving cry acoustics, these results suggest there is a biological underpinning to infant temperament.

The final set of results were in regard to a new questionnaire procedure developed for this project. This measure, self-efficacy in a nurturing role, was found to have good internal psychometric properties (alpha coefficients in the .80s), good reliability over a 5 month period with the vicissitudes of the birth of a baby (r = .63 for mothers), and meaningful but moderate overlap with a more generalized measure of the mother's emotional state, her anxiety level (r = -.41, prenatal period and r = -.49, postnatal period). The mother's accrual of experience with her baby was associated with a significant increase in self-efficacy in her nurturing role ($p < .001$), but her anxiety level appeared largely unchanged. One interesting hypothesis that remains to be tested is whether self efficacy relates to the mothers' reactivity patterns to the cry stimulus.

Analyses of data collected on both parents and infants through the end of the first year of life are currently underway. Although the Unit on Parent and Infant Studies will be formally disbanded with the retirement of Dr. Frank Pedersen, Drs. Huffman and del Carmen will continue the data analyes under the auspices of the LCE's Office of the Chief.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00054-15 LCE

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structural and Behavioral Analysis of Vocal Communication in Squirrel Monkeys

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Symmes	Head, BBCS	LCE, NICHD
Others:	M. Biben	Expert	LCE, NICHD
	D. Bernhards	Bio. Lab. Tech.	LCE, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Brain, Behavior, and Communication

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.6	1.8	.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our continuing interest in the communications systems of nonhuman primates has led us this year to studies of vocal development in infant squirrel monkeys. Nowhere do the theoretical biases of primatologists and child development experts diverge more clearly than in the problem of nature vs. nurture in primate language acquisition. The development of language skill in the human infant is viewed as enormously influenced by nurture while that of ape and monkey vocal communication is seen as driven primarily by genetic factors, a dichotomy obviously in the need of more study.

An understanding of the processes of vocal development in any species must necessarily begin from a base of knowledge of what is normal output for that species. We have recently begun an intensive study of vocal development in squirrel monkeys in captive but socially rich conditions, beginning the first week of life. Of particular interest to us are the ontogeny of affiliative calls which are used in responsive and variable ways and that have a slow course of development.

In preliminary investigations in FY88 we studied isolated mother-infant dyads to simplify the problem of identifying vocalizers. These mothers rarely vocalized to their infants, and youngsters, too, made few vocalizations. Only after we repeated the experiments with additional females in the cages did the auditory environment come to life. Newborns received a barrage of calls, mostly from females other than the mother. Most were "caregiver calls," a form of address which shows many similarities in form and usage to "motherese" in humans. The significance of aunts' caregiver calls became more clear when, in the first week of life, infants began to look toward the calling aunt and to utter vocalizations in return. We are currently analyzing this infantile vocal output, which represents the earliest instances of interactive vocal behavior in this species.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00062-13 LCE

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Physiological Control of Vocal Production in Squirrel Monkeys

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.D. Newman	Head, UN	LCE, NICHD
Others:	S. Boinski	NRSA Fellow	LCE, NICHD
	Y. E. Bryan	Visiting Fellow	LCE, NICHD
	L. Huffman	NRSA Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*
LCS, NIMH (Winslow); LN, NIMH (Bachevalier); Div. of Child Psychiatry and Dept. of Pediatrics, Johns Hopkins Sch. of Medicine (Harris)

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Unit on Neuroethology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates the pharmacological, neural, and physiological control of vocal behavior in well-characterized primate model species. Current work focuses on the isolation call, a characteristic vocalization that is the nonhuman primate homologue of human infant crying. One study completed this year revealed a difference in the modulatory role of monoamine oxidase inhibitors according to their primary neurotransmitter substrate on production of isolation calls in adult male squirrel monkeys. Another study revealed significant gender differences in the relationship of vagal tone and cry parameters in human infants at 3 months of age. In a third study, statistical analysis of separation coo vocalizations from rhesus macaques at 1 year of age revealed significant gender differences in some acoustic parameters, differences that were also present in subjects with neonatal bilateral ablations of the amygdala. A fourth study on juvenile rhesus monkeys demonstrated individual differences in immuno-reactivity that were related to certain acoustic parameters of coo vocalizations produced by these same subjects during the separation. Other studies on the vocal behavior of immature rhesus macaques demonstrated a correlation between the vocal behavior of infants with surgically corrected hydrocephalus and infants with bilateral ablations to the inferior temporal cortex.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00702-09 LCE

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetics of Primate Vocal Behavior

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. D. Newman	Head, UN	LCE, NICHD
Others:	S. H. Boinski	NRSA Fellow	LCE, NICHD
	J. Norcross	Guest Researcher	LCE, NICHD

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Unit on Neuroethology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is directed at identifying and differentiating heritable influences on vocal development in primates. Current work involves assessing the ontogenetic changes in acoustic structure of the primate isolation call, a vocalization given by individuals when separated from social companions. A field study of squirrel monkeys has demonstrated a significant correlation between the distance separating a vocalizer from other troop members and calling rate. In that study, the category of vocalizations containing the species-specific isolation call accounted for the greatest proportion of calls produced over distances exceeding 1 m. Another study involving captive juvenile rhesus macaques, found gender differences in the proportion of vocal subtypes produced during short periods of social separation, males producing more tonal "coo" sounds and females more atonal "leaps" and "screams." A related study with juvenile rhesus macaques found that the peak frequency and duration of "coos" produced by separated individuals gradually and systematically change with time since initial separation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01106-06 LCE

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Developmental Continuity of Individual Differences in Rhesus Monkey Reactivity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.J. Suomi	Head	LCE, NICHD
Others:	K.L. Rasmussen	IRTA Fellow	LCE, NICHD
	B. Black	NRSA Fellow	LCE, NICHD
	C.E. Eisele	Research Psychologist	LCE, NICHD

COOPERATING UNITS *(if any)* LDE, NICHD (Wheler, Loriaux); LCS, NIAAA (Linoilla, Higley); Primate Laboratory, Univ. Wisconsin-Madison (Coe, Schneider); Dept. of Obstet. & Gyn., Georgetown Univ. Med. Sch. (Michejda); Yerkes Reg. Primate Ctr. (Nadler, Bard); Istituto di Psicologia, CNR (Visalberghi); Univ. of Puerto Rico, Mayagez (Phoebus)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Comparative Behavioral Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	0.4	2.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates biobehavioral development through comparative longitudinal study of nonhuman primates, with special emphasis on characterizing individual differences among rhesus monkeys in response to mild environmental challenge and on determining the long-term developmental consequences for these individuals in different physical and social environments. Studies completed in FY89 further specified the interaction between genetic and environmental factors in shaping individual differences in response to challenge throughout development. Cross-fostering techniques permitted the demonstration that inherited predispositions toward extreme reactivity to challenge in young rhesus monkeys could be readily modified by certain types of maternal care, but that other patterns of maternal care were ineffective in changing the infants' characteristic responses. Modification of response style associated with the onset of puberty was also demonstrated in two independent studies. Parallel changes in behavior linked with physiological reactivity during adolescence was monitored in a group of adolescent males living in a natural troop of rhesus monkeys, with long-term outcome a function of the adolescents' characteristic response styles. Finally, neonatal tests designed to assess relative reactivity early in infancy were utilized to predict cognitive and social emotional developmental outcomes in juvenile rhesus monkeys and in two other nonhuman primate species.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01107-06 LCE

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Adaptation of Laboratory Reared Monkeys to Field Environments

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. J. Suomi	Head	LCE, NICHD
Other:	P. O'Neill	Research Psychologist	LCE, NICHD
	G. DiGregorio	Research Psychologist	LCE, NICHD
	C. Price	Biologist	LCE, NICHD

COOPERATING UNITS *(if any)*

VRB, DRS (Bayne); Department of Psychology, Univ. Massachusetts (Novak)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Comparative Behavioral Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	0.4	2.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

This project investigates how rhesus monkeys born and raised under different laboratory conditions adapt to placement into naturalistic outdoor environments and compares this adaptation process to that seen in natural settings and in indoor environments that contain specific physical and social features of the monkeys' natural habitat. Adaptation is assessed by examining behavioral repertoires and by monitoring a variety of physiological systems in these subjects, yielding broad-based indices of relative physical and psychological well-being. The project centers on longitudinal study of a group of 16-year-old rhesus monkeys and 2 generations of their progeny, all of whom live year-round in a 5-acre outdoor enclosure on the grounds of the NIHAC. Despite the fact that the 16-year-old adults were all laboratory born and hand-reared in a nursery, and that these adults and their progeny have never had physical exposure to any other monkeys, all members of this primary study group consistently exhibit the full compliment of species-normative behavioral repertoires, development patterns, seasonal changes (including well-defined breeding and birth seasons), and social organization. During FY89 these species-normative patterns continued to be documented in the primary study group, and comparisons with a second multigenerational group of laboratory-born rhesus monkeys maintained in indoor settings over a comparable period were extended. Two sets of studies investigating the effects of "enrichment" of the physical environment for rhesus monkeys living in different social settings were also completed in FY89. Finally, a procedure that involved systematically varying the location of highly preferred food items was developed to study foraging patterns and to test certain aspects of spatial memory and observational learning capabilities. A series of tests utilizing this foraging procedure was begun for two social groups of rhesus monkeys and tufted capuchin monkeys, respectively.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01108-05 LCE

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Comparative Studies of Play Behavior

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Biben	Expert	LCE, NICHD
Other:	D. Bernhards	Bio. Lab. Tech.	LCE, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Brain, Behavior, and Communication

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.4	.2	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Many questions about the motivation and control of play remain to be answered but are not amenable to our usual noninvasive techniques. We are currently investigating a more invasive approach employing various pharmacologic agents. Our studies this fiscal year have been limited to a pilot investigation of the effects of low doses of morphine on play in two young male monkeys, morphine being the drug most likely to increase play activity (Panksepp et al., 1984, 1985). Based on studies of the effects of morphine and naloxone, Panksepp and his coworkers have postulated the involvement of the central endogenous-opoid systems in the modulation of social play in juvenile rats. Both frequency and content of play are affected.

Play is a relatively fragile behavior which is easily disrupted by many stressors. Thus, we encountered an initial methodological problem in overcoming the stress of capture and injection. Subjects required several weeks of habituation to the procedures before they would play during the time period in which the drug was active. Subsequently we were able to induce them to play "on demand" by housing them separately and allowing them to be together, in a neutral cage, only during testing. We expect this to be a reliable experimental design for young males who are well acquainted with each other and apparently highly motivated to play when they are rejoined.

Because the specific ways in which morphine may affect play are not known, we have recorded our subjects' behavior on videotape so that, in addition to gross measures like frequency of

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01110-02 LCE

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Intuitive Parenting of Infants in Comparative Perspectives

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S.J. Suomi Chief, LCE LCE, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Child and Family Research Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Inactive

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 01111-04 LCE

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Factors Affecting Nurturant Behavior Toward Infants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	F. A. Pedersen	Head, UPIS	LCE, NICHD
Others:	Y. Bryan	Visiting Fellow	LCE, NICHD
	L. Huffman	NRSA Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*
University of Maryland (Porges); Brown University (Lester)

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Unit on Parent and Infant Studies

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
3.0	3.0	.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is concerned with emotional factors ascertainable in parents during the pregnancy period, reactivity to infant cries that have varying degrees of aversiveness, and the unique individuality of the infant as factors that collectively influence the course of the parent-infant relationship in the first year of life. First-time expectant mothers and their spouses were studied neonatally, and parents and infants were followed at 3, 9, and 12 months. Nonpregnant women were also studies with a subset of the procedures at times corresponding to the prenatal and 3 month periods. The study employs multiple levels of measurement, including observational, self-report, and physiological procedures. Data collection has been completed. Preliminary analyses, particularly for the prenatal and 3 month periods, have yielded several important findings. Among these are the following: (1) Physiological reactivity (change in heart rate) to the mildly stressful stimuli of recorded infant cries is attenuated among pregnant compared to non-pregnant women even though both groups are generally similar in their subjective ratings of cries with differing degrees of aversiveness. (2) Infant individuality was captured with biobehavioral markers early in life, including the acoustic properties of the infant's cry and a measure of heart rate variability; these indices show reliable associations with dimensions of infant temperament. (3) The appraisal of depression during pregnancy with a standard psychometric procedure, the Beck Depression Inventory, is sharpened by differentiating it into clusters that reflect cognitive-affective, reactivity, and somatic symptoms. This distinction highlighted differential patterns of change that tended to be obscured with the total Beck Depression Inventory. (4) A new questionnaire procedure was developed that measures parents' self-confidence in their nurturing skills. It shows good psychometric properties, meaningful relationships with other constructs, and is sensitive to the parents' accrual of experience with the infant. The various components of the investigation will converge in the appraisal of their contribution to the parent-infant attachment relationships at 12 months. Further analyses, especially the longitudinal course of measures, are being pursued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 01112-03 LCE

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effects of Home- and Out-of-Home Care on Child Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	M. E. Lamb	Head, SSED	LCE, NICHD
Others:	R. D. Ketterlinus	IRTA Fellow	LCE, NICHD
	K. J. Sternberg	Research Psychologist	LCE, NICHD

COOPERATING UNITS *(if any)* Center for Human Growth and Development, Univ of Michigan (F.L. Bookstein); Department of Psychology, Goteborg, Sweden (A. Broberg, C-P. Hwang); Department of Psychology, Catholic University (R. Cortez, M. Prodrinudus)

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Section on Social and Emotional Development

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.60	.30	.30

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project involves analyses of data from a longitudinal study in Sweden examining the effects of center day care, family day care, and home care on the development of 145 children recruited at an average of 16 months of age.

Multivariate analyses using Wold's Partial Least Squares "soft modelling" procedure have consistently indicated that type of care had no reliable impact on the children one and two years post-enrollment. In contrast, the quality of care received at home and the quality of alternative care had an equivalent impact on personality maturity and emergent social skills with peers and adults. Measures of family social support networks, temperament, and child gender had more modest effects. PLS analyses also showed that quality of home care was the most important predictor of intellectual competence one and two years after enrollment. Compliance with maternal requests in a task-like situation was most strongly predicted by the quality of care received at home. The quality and extent of alternative care were also significant predictors of compliance.

A second study in this project involves a small but intensive investigation of family day care in Utah. Data from this study suggest that while it is possible to obtain reliable assessments of peer and careprovider interactions, quality of care is only marginally associated with social skills in toddlers. A third study focuses on the association between infant daycare and security of infant-mother attachment in more than a dozen studies conducted by other investigators. Analyses suggest that there is no systematic relationship between enrollment in daycare and security of attachment.

These findings all underscore the need to consider not only the type but also the quality of out-of-home care, and to consider the role of factors outside the care setting--such as the quality of home care--when evaluating day care arrangements.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 01113-03 LCE

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Antecedents, Correlates, and Consequences of Adolescent Pregnancy and Parenthood

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.E. Lamb Head, SSED LCE, NICHD
Other: R.D. Ketterlinus IRTA Fellow LCE, NICHD

COOPERATING UNITS *(if any)*
Dept. of Psychology, U of MD-Baltimore County (Teti, Das); Dept. of Pediatrics, U of Utah Med. Sch. (Elster); Dept. of Human Dev., U of MD (Holloway, Kimmerly); Dept. of Psychology, Catholic U (Henderson); Dept. of Psychology, U of VA (Gardner).

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Section on Social and Emotional Development

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goals of this project are to describe the psychosocial context of adolescent parenthood and to explore the long term effects for mothers, fathers, and children. Our most recent work has involved analysis of data from large nationally representative samples that address two basic questions.

A. Characteristics of adolescent parents. Regardless of race, adolescent parenthood was found to be one symptom of a wide variety of psychosocial problems. Compared with nonfathers and nonmothers of similar ages and backgrounds, adolescent parents are more likely to have a history of involvement with the police, school problems, and substance abuse. A syndrome of problem behaviors is especially marked among adolescent men.

B. Long-term correlates of adolescent parenthood. Adolescent marriage is associated in men and women with deficits in marital stability, income, educational attainment, and occupational prestige up to 40 years after the marriage. The "best" outcomes are obtained by those women who delayed both childbearing and marriage into adulthood. Younger adolescent mothers are lighter, gain less weight during pregnancy, seek prenatal care later in their pregnancies, and tend to be from lower socioeconomic classes as compared to older adolescent and adult mothers. Children of very young adolescent mothers were found to be at risk for low birthweight and premature birth. Among black and Hispanic adolescent and adult mothers maternal age is not associated with children's cognitive performance at ages 5-14. For whites, the children of very young adolescent women exhibit the poorest cognitive performance. Overall, maternal intelligence and socioeconomic status factors are better predictors of children's performance than is mother's age.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 01114-02 LCE

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Individual Differences in Physical and Affective Functioning in Infancy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.E. Lamb	Head, SSED	LCE, NICHD
Other:	A. Rosenberg	IRTA Fellow	LCE, NICHD
	R.D. Ketterlinus	IRTA Fellow	LCE, NICHD
	M.P. Fracasso	IRTA Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*
Department of Psychology, U. of Maryland (S.J. Porges); Department of Psychology, Catholic U. (D. Haynie);

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Section on Social and Emotional Development

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study is concerned with the ways certain physiological and behavioral signs of arousal or irritability (e.g., heart rate, heart rate variability, vagal tone, colic, sleeplessness, crying) in the first 5 months of life are related to measures of the child's temperament, emotional expressiveness, and physiology at later ages. By observing patterns of infant-mother interaction both at home and in laboratory settings, we further expect to determine whether individual differences in maternal behavior interact with early physiological and temperamental patterns in determining psychophysiological functioning, emotional expressiveness, attachment, and behavioral inhibition in toddlerhood.

Preliminary results from the initial (5 month) phase of the study indicated that infants described at 4 months of age as extremely irritable scored higher than nonirritable babies on the fussy and unadaptable subscales of the ICQ when they were 5 months old. Irritable babies were more likely to have extremely high or low vagal tone, and extremely high or low heart rate, than were nonirritable babies. Additionally, children with extremely high or low vagal tone or heart rate appeared more fussy and unadaptable on the relevant ICQ subscales.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 01115-02 LCE

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Effects of Domestic Violence on Children's Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.E. Lamb	Head, SSED	LCE, NICHD
Other:	K.J. Sternberg	Research Psychologist	LCE, NICHD

COOPERATING UNITS *(if any)*
Catholic U. (Cortez, Krispin); Hebrew U., Jerusalem (Greenbaum, Limor); Jerusalem Municipality (Garber, Lorey, Saltzman, Zeek); U of Maryland (Sandler); U of North Dakota (Lewnsohn); U of Rochester (Cicchetti)

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Section on Social and Emotional Development

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.70	.20	.50

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to explore the effects of domestic violence on 10- to 12-year-old children. The study involves four groups of subjects, each comprising 15 boys and 15 girls, defined by whether they have been (1) the victims of physical abuse by their fathers; (2) the witnesses of physical abuse of their mothers by their fathers; (3) both victims and witnesses of domestic violence by their fathers; and (4) children from similar backgrounds who have not experienced any forms of domestic violence. Data are being obtained from the children, their parents, their teachers, and their peers. The focus is on the quality of the children's functioning at home, at school, and in the peer group, with attempts made to explore the intrapersonal (temperament; perceptions of responsibility and control) and exogenous (social support) factors that buffer some children while rendering others more vulnerable. Data collection took place (under contract) in Israel from August 1988 to October 1989. This is one of the first methodologically sound studies comparing the effects of various types of domestic violence on children's development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 01116-02 LCE

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Patterns of Childrearing Across Cultures and Ecologies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	M.E. Lamb	Head, SSED	LCE, NICHD
Others:	A.B. Nsamenang	Fogarty Visiting Fellow	LCE, NICHD
	K.J. Sternberg	Research Psychologist	LCE, NICHD
	M.H. Van Ijzendorn	Fulbright Visiting Fellow	LCE, NICHD

COOPERATING UNITS *(if any)* U. Osnabruck, West Germany (H. Keller, H-G Voss); U. Goteborg, Sweden (A. Broberg, C.-P. Hwang); U. of NC, Greensboro (C. MacKinnon); U. Maryland (D. Teti, M. Nakagawa); Institute of Human Sciences, Cameroon (P.B. Soh); Hebrew U., Jerusalem (C. Greenbaum, M. Rosenthal); Catholic U. (R. Cortes).

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Section on Social and Emotional Development

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.63	1.53	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The work on this project involves a number of studies in a variety of cultures. The overall objective is to explore the ways developmental environments can be described by variations in physical and social ecologies, especially in terms of parental beliefs and values, parenting programs, and how differences in these domains affect children's development. In the first study, SSED staff collected interview data about perceptions, values, expectations, responsibilities, and practices of West African parents. These data are being analyzed and will be interpreted in the context of information about variation in developmental niches. In a second study, SSED staff explored the effects of agreement between Swedish mothers and fathers regarding socialization values. Agreement between parents was less clearly associated with development outcomes in Sweden than in the USA. In the third study, SSED staff are attempting to assess specific maternal and child attributions about one another in order to identify the extent to which attributions or expectations shape the way that parents and children interact. Finally, SSED staff are exploring the validity of Strange Situation assessments of infant-mother attachment security in Japanese dyads.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 01117-02 LCE

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Hospitalization Experience: Children's Coping with the Stress of Surgery

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.H. Bornstein Head LCE, NICHD

COOPERATING UNITS *(if any)*

Department of Psychology, New York University (Altshuler)

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Section on Child and Family Research

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.1	.1	.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This research is designed to examine age differences in children's understanding of and reactions to a brief stay in the hospital for elective surgery. The research integrates the adult stress and coping literature with that on changes in children's cognitive capabilities as they mature. Two experiments have been conducted, one on children's opinions and a second on children's reactions before hospitalization, on the threshold of surgery, and after hospitalization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 01118-02 LCE

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Latent Behavioral Effects of Diverse Forms of Caretaking in the First Year of Life

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.H. Bornstein	Head	LCE, NICHD
Other:	N.F. Gist	Research Psychologist	LCE, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Section on Child and Family Research

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	.2	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This research study is designed to explore the latent effects of different kinds of care in infancy on preschool children's mental and social performance. Mothers entered the workforce before their children were 12 months of age. This study is the first in a series of investigations preliminary to a large-scale endeavor to document the effects of varied rearing conditions in the first year of life on children's activities and competencies at preschool age. Data are being gathered on a homogeneous, low-risk population and include measures of cognitive, social, and behavioral development.

Plans are also being formulated to obtain additional developmental and behavioral data on the children just prior to completion of first grade. This will allow us to compare academic achievement with earlier cognitive status, and to compare children's social behaviors at the two points in time.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01119-02 LCE

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Specificity of Mother-Infant Interaction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.H. Bornstein	Head	LCE, NICHD
Others:	J. Suwalsky	Research Psychologist	LCE, NICHD
	P. Ludemann	Research Psychologist	LCE, NICHD
	M. Fivel	Research Psychologist	LCE, NICHD
	C. Rahn	Research Psychologist	LCE, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Child and Family Research

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	.2	3.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates environmental factors that contribute to the development of cognitive competencies during the first two years of life. Before children are old enough to enter formal social learning situations, nearly all of their experiences stem directly from interactions they have with their primary caretakers. Four conceptually distinct categories of caretaker-child interactions can be identified: nurturant, material, social, and didactic. These encompass much of the everyday behavior of infants' caretakers. In previous work using samples of convenience, the Principal Investigator linked the latter two types of behavior to cognitive development in babies. In the present study set, this work is being replicated and extended by focusing on the extent to which three maternal characteristics (age, employment status, and parenthood status) and type of substitute care experienced during mother's employment influence the observed relations between caregiver social and didactic stimulation on the one hand and infant social and cognitive competencies on the other. In addition, in a short-term longitudinal extension of the study, measures of toddler functioning (i.e., play competence, language development, and social adaptation) and maternal behavior (i.e., encouragement of attention to the environment and I.Q.) will be obtained, thereby permitting examination of associations between aspects of mother-infant interaction in early infancy and the development of important aspects of cognitive and social competence in the second year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 01120-02 LCE

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Observations of Parenting and Infant Activity in Different Societies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.H. Bornstein	Head	LCE, NICHD
Other:	S. Toda	Visiting Fellow	LCE, NICHD
	P. Ludemann	IRTA Fellow	LCE, NICHD
	C. Rahn	Research Psychologist	LCE, NICHD

COOPERATING UNITS *(If any)*
Haifa School Psychological Services, ISRAEL (Maital); Universidad de Belgrano, ARGENTINA (Zingman de Galperin); Shirayuri College, JAPAN (Azuma); Laboratoire de Psychologie du Développement et de l'Éducation de l'Enfant, FRANCE (Pêcheux)

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Section on Child and Family Research

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Experiences in infancy are acknowledged to affect social and intellectual development, and they are credited for some of the distinctiveness of different cultures. More specifically, cross-cultural developmental studies have shown historically that differences in rearing typically have implications for children's later cognitive performance and social behavior. Home environments are thought to reflect larger cultural values, beliefs, and customs, and many social theorists have contended that the family generally, and the mother-infant relationship specifically, may be vital to development of the individual and basic to the organization of the culture. As a result, investigators have frequently studied infancy and mother-infant interaction in attempts to address questions about the origins and development of cultural identity. Of course, each society has evolved patterns of child-rearing adjusted to its own special demands.

It is widely held, for example, that Japanese and Americans differ in prominent aspects of their psychological make-ups and that certain social and intellectual distinctions between members of these two cultures arise early in life. Previous study on the nature of infant development in Israeli Kibbutzim determined that many decisive aspects of infant care -- particularly the close ties between infants and mother -- vary markedly from the typical American experience. Contemporary France and America are relatively similar in terms of industrial level, educational attainment, and living standards, yet the two societies differ considerably in terms of history, sociology, and culture. Argentina contrasts middle with extremely poor rearing conditions, all in South American settings virtually unresearched. What differences exist in parenting and in infant activity in these cultures? The purpose of this project is to identify similarities and differences in the childrearing ecologies of Japanese, Israeli, French, Argentinean, and American infants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 01121-02 LCE

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Parental Activities and Children's Language and Play

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.H. Bornstein Head LCE, NICHD

COOPERATING UNITS *(if any)*

Harlem Hospital Center (Vibbert)

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Section on Child and Family Research

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.2	.2	.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This research project investigates concurrent and longitudinal contributions of maternal and paternal activity to toddler language and play competencies in the second year of life. Domains of parental activity include didactic attention focusing, interpersonal affective communication, and control over object-centered exchanges. Several major data sets have been collected on parental style, on toddler competencies, and on the independent and joint contributions of parental style to toddler abilities. Each of these data sets is multifold, and analyses of results have formed the bases for several different, but related, projects to be described herein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01122-02 LCE

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Assessment of Children's Mental and Social Abilities

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.H. Bornstein	Head	LCE, NICHD
Other:	C. Tamis-LeMonda	IRTA Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*

Program in Applied Child Development, Tufts University (Feldman)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Child and Family Research

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.7	.7	.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main purpose of this research is to continue and to followup an ongoing longitudinal study in New York City. The premise of this research is that learning in childhood occurs primarily within an interpersonal context; thus, a central goal is to describe diverse maternal behaviors in relation to central dimensions of children's social and mental competencies. One aspect of the follow-up is to test materials based on the curriculum-oriented Project Spectrum, organized between the Harvard University School of Education and the Eliot-Pearson Department of Child Study at Tufts University. Project Spectrum is unique in the United States for its development of a curriculum that goes far beyond IQ to assess a wide range of preschool children's interests and capabilities. The "multiple intelligences" that Project Spectrum's procedures assess include natural science ability, bodily-kinesthetic skills, musical talents, and distinctive styles of work, as well as linguistic and logical-mathematical abilities. Because traditional psychometric measures of intelligence at this age sample from a narrow range of mental abilities, such measures are limited in terms of the information they provide about the possible relevance of antecedent variables and are also restricted in terms of the outcome variables they successfully predict. In contrast, the Project Spectrum Field Assessment Battery samples from a wide range of preschoolers' cognitive capabilities and interests and thus affords richer opportunities to respond to many theoretical and pragmatic questions that surround the central issue of stability in mental development.

LABORATORY OF DEVELOPMENTAL AND MOLECULAR IMMUNITY (LDMI)

Z01 HD 00073-18	Regulation of Immune Systems at the Cellular Level Edgar E. Hanna, Ph.D.
Z01 HD 01301-07	Human Immune Response to Polysaccharide-Protein Conjugate Vaccines Rachel Schneerson, M.D.
Z01 HD 01304-07	Protective Effect of Vi and LPS Antibodies Against Typhoid and Shigellae John B. Robbins, M.D.
Z01 HD 01307-06	Pertussis Toxin: An Approach to a New Pertussis Vaccine John B. Robbins, M.D.
Z01 HD 01308-06	Conjugation of Pneumococcal Staphylococcus Aureus and Vi Polysaccharides with Proteins Shousun C. Szu, Ph.D.
Z01 HD 01310-03	Developmental Gene Regulation of the Immune System Keiko Oazto, Ph.D.

Laboratory of Developmental and Molecular Immunity

The LDMI is concerned with immunologic development with especial interest in its relation to infectious diseases of neonates and young children.

The Section on Bacterial Disease Pathogenesis and Immunity (John Robbins) is working on the problem of the synthesis and protective actions of conjugate vaccines composed of capsular polysaccharides and de-lipidated LPS. This work was stimulated by the age-related and T-independent properties of Haemophilus influenzae type b capsular polysaccharide (Hib), and other polysaccharides of invasive bacteria, limit their protective actions in infants and children, that age group with the highest attack rate of diseases due to these pathogens. An organic synthetic scheme, that bound Hib and other capsular polysaccharides to carrier proteins in a clinically-acceptable manner, were devised in order to both increase the immunogenicity of and confer T-cell dependence (booster effect) to these protective antigens. A conjugated Hib vaccine, prepared by the original method of the LDMI, was licensed by the FDA for universal use in children older than 18 months of age. The safety and immunologic properties of our Hib-TT vaccine have been investigated in 18-23 month olds and now in 2-3 month old infants. One injection of Hib-TT into 18-23 month old children elicited protective levels of antibodies in all recipients. One year following the injection these antibodies were statistically higher than the age-related development of Hib antibodies in that group. Three injection of Hib-TT to infants in Goteborg, Sweden and in Charlotte, N.C., showed that protective levels were reached in almost all, after two injections and in all after three injections. Neutralizing antibodies to tetanus toxoid, the carrier protein, were also elicited by these vaccines. The same technology was applied to preparing conjugates of Pneumococcus type 6 bound to tetanus toxoid. This pneumococcus conjugate vaccine was shown to be statistically more immunogenic in 2-5 year-old Sickle Cell Anemia patients than the Pneumococcus type 6 polysaccharide alone. The conjugate elicited protective levels of antibodies in 96% of the recipients compared to none of the age-related patients injected with the type 6 polysaccharide. Similar results have been obtained with conjugates of Penumococcus types 12F in volunteers and 14 in mice. New methods of synthesis for group B meningococcus capsular polysaccharide (also E. coli K1) to form conjugates are under study.

Covalent attachment of polysaccharides to form optimal conjugate vaccines, is dependent upon both the size and structure of the two components. Methods for conjugating capsular polysaccharides, including the Vi capsular polysaccharide and pneumococcus types 6B, 12F, 19F, and 23F were devised using principles previously established with new joining reagents. Polysaccharides having carboxyl groups, single hydroxyl or vicinal hydroxyl groups were treated with different cross-linking reagents prior to their conjugation with carrier proteins. Dextran was used as a model polysaccharide for study. Bacterial polysaccharides tested were: Vi, pneumococcus types 6B, 12F, 19F, 19A, and 23F, O-specific side-chains of Shigella dysentariae type 1, Salmonella paratyphi A, Staphylococcus aureus types 5 and 8. The Vi was modified by O-deacetylation and decarboxylation in order to study the relationship between structure and immunogenic epitopes. The immunogenicity of Vi conjugates, prepared with polysaccharides of large and low

molecular weights and with the beta subunit and holotoxin of cholera toxin were compared in laboratory animals. In addition, clinical studies of a Vi-tetanus toxoid conjugate in adults were started.

The incidence and severity of pertussis (whooping cough) have been controlled by immunization with inactivated Bordetella pertussis organisms (cellular vaccine) as the P component of DTP. The identification of pertussis toxin, as a major, if not the sole protective antigen for B. pertussis, has led to the development of a toxoid derivative of this antigen. Pertussis toxin was purified by affinity chromatography from the culture supernant of B. pertussis and inactivated by hydrogen peroxide under controlled conditions. The resultant product, called NICHD-PTxD, had less than 1% of its original binding and enzymatic activities and showed no pharmacologic properties of the holotoxin in in vivo assays such as the histamine sensitization test. Clinical evaluation of the first clinical lot of this toxoid vaccine in adults, and later in 18-month old children showed it to be safe and more immunogenic than the cellular pertussis vaccine component. Antibodies induced by the toxoid neutralized the biologic properties of the holotoxin (antitoxin). The toxoid-induced IgG antibodies were almost exclusively, of the IgG1 subclass but some in the IgG4 subclass. Clinical evaluation of the second clinical lot of the toxoid in infants, injected at either three, five and seven, or three, five and twelve months according to the recommended schedule in Sweden, showed no local or systemic reactions confirming its superior safety characteristics. The serological response to this investigational vaccine is now under study.

The Section on Molecular Genetics of Immunity (Keiko Ozato) is studying the mechanisms of gene regulation in the developing immune system with a major emphasis on transcriptional control of the MHC class I genes. MHC class I genes encode a series of polymorphic transplantation antigens that regulate the development and functions of the immune system. In the 5' upstream region there is a conserved cis-regulatory complex that controls transcription of MHC class I genes. This complex contains the class I regulatory element and interferon consensus sequences (CRE and ICS). At least two separate nuclear factors bind to two different regions of the CRE, which result in the enhanced transcription of the MHC genes. A factor induced by IFN induces transcription of the MHC genes following interferon treatment. MHC transcription is repressed in early embryonic cells and this repression is also governed by the CRE-ICS complex. A nuclear factor that binds to this negative regulatory site has been detected in embryonal carcinoma cells, but not other cells that does not show the MHC gene repression. These findings show that the conserved CRE-ICS complex and factor binding are control mechanisms that govern transcription of the MHC genes. Regulatory genes are being sought by screening phage expression libraries using CRE and ICS as probes. This method relies on in situ DNA-protein interactions in order to isolate factor cDNA without information on the detailed protein structures. Two cDNA clones, that encode proteins capable of binding to the MHC regulatory complex, have been isolated. These clones, called H-2RIIBP and ICSBP, bind the region II of the CRE and the ICS respectively. H-2 RIIBP is composed of a Zn finger and modular domains characteristic of the steroid/thyroid hormone receptors. H-2RII BP is expressed widely in mouse tissues, and conserved among many animal species including Drosophila. The role of c-fos in regulatory MHC expression is being studied with antisense RNA as specific inhibitors.

The Section on Immunoregulation and Cellular Control (Edgar Hanna) studies the

mechanisms by which micro-organisms modify the immune systems. We postulate this modulation is mediated during the developmental and regulatory processes of regulatory T-cells for antibody forming cells and cytotoxic T-cells. Macrophages and natural cytotoxic (NK) cells may also be effected through effects upon their regulatory cell precursors. Various bacterial toxins were used as probes to delineate this mechanism. Using an in vitro modular immune system ("cell complementation system" involving murine spleen immunocytes) which allows us to rearrange the order and relative numbers of toxin-treated/or untreated cells when recombined in the immune system. Perpetual cell lines were cloned possessing the functions of many of the native regulatory immunocytes. These phenocopies of regulatory cells are used as targets for the bacterial toxins and subsequently tested for altered function. These clones may be stored and used as homogenous cells at representative stages of development. We have selected suppressor(-) clones from one of our suppressor precursor clones (NBP2C2, a CD4+/CD8+ clone), in the presence of SPE. Whereas, clones selected similarly using Et or LPS resulted in subclones retaining the parental phenotype. Cell-free supernatants of Et treated, NFR/N or nude mouse splenocytes supported an 80-90% recovery of suppressive activity by the SPE selected subclone. Macrophage-depletion negated this activity of supernatants. Selected clones were observed not to differ from their parent in expression of MHC haplotype and expression of an epitope of the TCR-agn. Suppression of the expression of IL-2R in the parent clone by SPE and variously to smaller magnitudes by TSST-1, Pt, and SE-C. The SPE selected subclone expressed markedly less CD8 than its parent. These results have been confirmed and are proposed to largely explain the contra-suppressive activity of SPE and similar bacterial toxins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00073-18 LDMI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Immune Systems at the Cellular Level

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: E. Hanna Head, SICC LDMI, NICHD

COOPERATING UNITS *(if any)*

LN, NIDDK, P. Arora; OGC, NICHD, M. Walker; VR, DRS, C. Hansen

LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

SECTION

Section on Immunoregulation and Cellular Control

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland, 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This laboratory is pursuing an understanding of the mechanisms by which micro-organisms activate, modulate, or subvert immune systems. We postulate these events are mediated during the developmental and regulatory processes of regulatory T-cells for antibody forming cells and cytotoxic T-cells. Macrophages and natural cytotoxic (NK) cells may also be effected through modulatory effects upon their precursors of their regulatory cells. We have exploited various bacterial toxins as natural probes to facilitate an experimental delineation of mechanisms in this respect. Using an in vitro modular immune system ("cell complementation system" involving murine spleen immunocytes) which allows us to rearrange the order and relative numbers of toxin treated/or untreated cells when recombined in the immune system. Further progress was made by constructing and cloning perpetual cell lines possessing the functions of many of the native regulatory immunocytes. These phenocopies of regulatory cells are used as targets for the bacterial toxins and subsequently tested for altered function. Because these clones may be stored and revived at will cryogenically, they promote continuity of on-going experiments in using homogenous cells at representative stages of development. We have selected suppressor(-) clones from one of our suppressor precursor clones (NBP2C2, a CD4+/CD8+ clone), in the presence of SPE. Whereas, clones selected similarly using Et or LPS resulted in subclones retaining the parental phenotype. Cell-free supernatants of Et treated, NFR/N or nude mouse splenocytes supported an 80-90% recovery of suppressive activity by the SPE selected subclone. Macrophage-depletion negated this activity of supernatants. Selected clones were observed not to differ from their parent in expression of MHC haplotype and expression of an epitope of the TCR-agn. Suppression of the expression of IL-2R in the parent clone by SPE and variously to smaller magnitudes by TSST-1, Pt, and SE-C. The SPE selected subclone expressed markedly less CD8 than its parent. These results have been confirmed and are proposed to largely explain the contra-suppressive activity of SPE and similar bacterial toxins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01301-07 LDMI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Human Immune Response to Polysaccharide-Protein Conjugate Vaccines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Schneerson	Research Medical Officer	LDMI, NICHD
Others:	J.B. Robbins	Head	LDMI, NICHD
	S. Devi	IRTAFellow	LDMI, NICHD
	L. Levi	Chemist	LDMI, NICHD
	T. Cramton	Chemist	LDMI, NICHD

COOPERATING UNITS *(if any)* SUNY Downstate Medical Center, N.Y., G. Shiffman; Charlotte Memorial Hospital, N.C., J.C. Parke, Jr.; USUHS, Bethesda, M.D., J. Schesselman; University of Goteborg, Sweden, T. Lagergard, B. Trollfors, J. Taranger, B. Claesson; OD, NICHD, C. Lowe; PRP, NICHD, D. Bryla.

LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

SECTION

Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland, 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.4	1.9	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The age-related and T-independent properties, of Haemophilus influenzae type b capsular polysaccharide (Hib) and other polysaccharides of invasive bacteria, limit their protective actions in infants and children, that age group which the highest attack rate of diseases due to these pathogens. Organic synthetic scheme, that bound Hib and other capsular polysaccharides to carrier proteins, were devised in order to both increase the immunogenicity of and confer T-cell dependence (booster effect) to these protective antigens. Based upon ours, and others work in the field, a conjugated Hib vaccine, prepared by our original method, was licensed by the FDA for universal use in children older than 18 months of age. The safety and immunologic properties of our Hib-TT vaccine have been investigated in 18-23 month olds and now in 1-3 month old infants. One injection of Hib-TT into 18-23 month old children elicited protective levels of antibodies in all recipients. One year following the injection these antibodies were statistically higher than the age-related development of Hib antibodies in that group. Three injection of Hib-TT to infants in Goteborg, Sweden and in Charlotte, N.C., showed that protective levels were reached in almost all, after two injections and in all after three injections. Neutralizing antibodies to tetanus toxoid, the carrier protein, were also elicited by these vaccines. New methods of synthesis for group B meningococcus capsular polysaccharide (also E. coli K1) to form conjugates are under study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01304-07 LDMI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protective Effect of Vi and LPS Antibodies Against Typhoid and Shigellae

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.B. Robbins	Head	LDMI, NICHD
Others:	R. Schneerson	Research Medical Officer	LDMI, NICHD
	C. Chu	Visiting Scientist	LDMI, NICHD
	E. Daniels	NRSA Fellow	LDMI, NICHD
	B. Liu	Adjunct Scientist	LDMI, NICHD
	T. Cramton	Chemist	LDMI, NICHD
	N. Tolson	Biologist	LDMI, NICHD

COOPERATING UNITS *(if any)*

Institut Merieux, France, M. Cadoz; Agency for International Development, Washington, D.C., F.-Y.C. Lin; OD, NICHD, C. Lowe; PRP, NICHD, D. Bryla, J. Rigau.

LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

SECTION

Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland, 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.2	3.9	1.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Enteric fevers remain a serious and frequent cause of morbidity and mortality throughout the World. These group of intestinal diseases are caused by the genus Salmonellae. The epidemiology and frequency of enteric fevers differ throughout the World; the most frequent and serious of these enteric fevers in underdeveloped nations is typhoid fever caused by Salmonella typhi. The next most common cause of enteric fevers in underdeveloped nations is S. paratyphi A. In the United States, Salmonellae of the groups B and D are the most common causes. Of these, S. typhimurim and S. enteridiidis are the most frequent species encountered in human diseases. Evaluation of vaccines for prevention of these diseases has a long and varigated history because the most Salmonellae are inhabitants of and pathogens for humans only. Two, double-masked, randomized, controlled evaluations of the Vi capsular polysaccharides of S. typhi has shown its ability to prevent typhoid fever in Nepal and in the Eastern Transvaal of the Republic of South Africa. Effectiveness rates of about 70% were observed in both areas that have high attack rates of this disease. No significant side reactions were observed. The effectiveness of the Vi has declined in both sites in the third year. Surveillance and long-term serologic studies have provided an estimate for the protective level of vaccine induced Vi antibodies. Based upon these data, new vaccines for the prevention of non-typhoidal enteric fevers, using the model of the Vi capsular polysaccharide conjugates, is under study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01307-06 LDMI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pertussis Toxin: An Approach to a New Pertussis Vaccine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.B. Robbins	Head	LDMI, NICHD
Others:	R. Schneerson	Research Medical Officer	LDMI, NICHD
	N.W. Tolson	Biologist	LDMI, NICHD

COOPERATING UNITS *(if any)*

LCDB, NIDDK, J. Shiloach, B. Kaufman; University of Goteborg, Sweden, T. Lagergard, B. Trollfors, J. Taranger; PRP, NICHD, J. Rigau, D. Bryla; OD, NICHD, C. Lowe.

LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

SECTION

Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland, 20892.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The incidence and severity of pertussis (whooping cough) have been controlled by immunization with inactivated Bordetella pertussis organisms (cellular vaccine) as the P component of DTP. The identification of pertussis toxin, as a major, if not the sole protective antigen for B. pertussis, has led to the development of a toxoid derivative of this antigen. Pertussis toxin was purified by affinity chromatography from the culture supernant of B. pertussis and inactivated by hydrogen peroxide under controlled conditions. The resultant product, called NICHD-PTxD, had less than 1% of its original binding and enzymatic activities and showed no pharmacologic properties of the holotoxin in in vivo assays such as the histamine sensitization test. Clinical evaluation of the first clinical lot of this toxoid vaccine in adults, and later in 18-month old children showed it to be safe and more immunogenic than the cellular pertussis vaccine component. Antibodies induced by the toxoid neutralized the biologic properties of the holotoxin (antitoxin). The toxoid induced IgG antibodies almost exclusively, mostly of the IgG1 subclass but some in the IgG4 subclass. Clinical evaluation of the second clinical lot of the toxoid in infants, injected at either three, five and seven, or three, five and twelve months according to the recommended schedule in Sweden, showed no local or systemic reactions confirming its superior safety characteristics.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01308-06 LDMI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Conjugation of Pneumococcal, Staphylococcus aureus and Vi Polysaccharides with Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.C. Szu	Research Chemist	LDMI, NICHD
Others:	J.B. Robbins	Head	LDMI, NICHD
	A. Fattom	Visiting Associate	LDMI, NICHD
	D. Watson	NRC Fellow	LDMI, NICHD
	U. Sorensen	Adjunct Scientist	LDMI, NICHD
	X. Li	Visiting Fellow	LDMI, NICHD
	T. Cramton	Chemist	LDMI, NICHD

COOPERATING UNITS *(if any)*

LI, NIAID, J.K. Inman; DBP, CBER, W. Vann; Pennsylvania State University, PA, W. Karakawa.

LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

SECTION

Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland, 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.6	5.1	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Covalent attachment of polysaccharides that are potentially capable of eliciting protective antibodies, to carrier proteins to form optimum conjugate vaccines, is dependent upon both the size and structure of the two components. Methods for conjugating capsular polysaccharides, including the Vi capsular polysaccharide and pneumococcus types 6B, 12F, 19F, and 23F were devised using principles previously established in this and other laboratories as well as new joining reagents. The methods for conjugating polysaccharides of different chemical characteristics were developed. Polysaccharides having carboxyl group, single hydroxyl or vicinal hydroxyl groups were treated with different cross-linking reagents prior to their conjugation with carrier proteins. Dextran was used as model polysaccharide for study. Bacterial polysaccharide tested were: Vi, pneumococcus types 6B, 12F, 19F, 19A, and 23F, O-Specific side-chains of Shigella dysentariae type 1, Salmonella Paratyphi A, Staphylococcus aureus types 5 and 8. The Vi was modified by O-deacetylation and decarboxylation in order to study the relationship between structure and immunogenic epitopes. The immunogenicity of Vi conjugates, prepared with polysaccharides of large and low molecular weight and with the beta subunit and holotoxin of cholera toxin were compared in laboratory animals. In addition, clinical studies of a Vi-tetanus toxoid conjugate in adults were started.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01310-03 LDMI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Developmental Gene Regulation of the Immune System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: K. Ozato Head, SMGI LDMI, NICHD

See Attached

COOPERATING UNITS *(if any)*

LCB, NCI, E. Appella; LDP, NICHD, A. Dey, D. Nebert.

LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

SECTION

Section on Molecular Genetics of Immunity

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland, 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
9.6	8.3	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This program addresses the mechanisms of gene regulation in the developing immune system. Transcriptional control of the MHC class I genes is the major emphasis. The MHC class I genes encode a series of polymorphic transplantation antigens that play a important role in the development and functions of the immune system. In the 5' upstream region there is a conserved cis-regulatory complex that controls transcription of MHC class I genes. This complex contains the class I regulatory element and interferon consensus sequence (CRE and ICS). At least two separate nuclear factors bind to two different regions of the CRE, which result in the enhanced transcription of the MHC genes. The ICS, by binding to a factor that is induced by IFN induces transcription of the MHC genes following interferon treatment. In addition, MHC transcription is repressed in early embryonic cells and this repression is also governed by the CRE-ICS complex. A nuclear factor that binds to this negative regulatory site has been detected in embryonal carcinoma cells, but not other cells that does not show the MHC gene repression. These findings show that the conserved CRE-ICS complex and factor binding to it constitute the central mechanisms governing transcription of the MHC gene. In order to isolate regulatory genes, we screened phage expression libraries by using CRE and ICS as probes. This method that relies on in situ DNA-protein interactions allows isolating factor cDNA without information on the detailed protein structures. We isolated two cDNA clones that encode proteins capable of binding to the MHC regulatory complex. These clones, called H-2RIIBP and ICSBP, bind the region II of the CRE and the ICS respectively. H-2 RIIBP is composed of a Zn finger and modular domains characteristic of the steroid/thyroid hormone receptors. H-2RII BP is expressed widely in mouse tissues, and conserved among many animal species including Drosophila.

Others:	J. Flanagan	Senior Staff Fellow	LDMI, NICHD
	B.-Z. Levi	Visiting Associate	LDMI, NICHD
	S. Hirschfeld	Medical Staff Fellow	LDMI, NICHD
	P. Driggers	IRTA Fellow	LDMI, NICHD
	S. Gleason	NRC Fellow	LDMI, NICHD
	D. Ennist	NRC Fellow	LDMI, NICHD
	K. Hamada	Visiting Fellow	LDMI, NICHD
	Y. Shirayoshi	Visiting Fellow	LDMI, NICHD
	K. Becker	IRTA Fellow	LDMI, NICHD
	W.-H. Mak	Chemist	LDMI, NICHD
	K. Phimmascone	Bio-Aid	LDMI, NICHD

LABORATORY OF DEVELOPMENTAL NEUROBIOLOGY (LDN)

Z01 HD 00047-20	Biochemical Studies of Neuronal and Other Cell Types Douglas E. Brenneman, Ph.D.
ZO1 HD 00056-14	Biosynthesis, Processing & Secretion of Neuropeptides & Pituitary Peptide Hormones Y. Peng Loh, Ph.D.
Z01 HD 00064-13	Neurobiologic Studies of Neurons and Glia in Cell Culture Phillip G. Nelson, M.D., Ph.D.
Z01 HD 00094-19	Pineal Regulation: Environmental and Physiological Factors David C. Klein, Ph.D.
Z01 HD 00095-19	Pineal Regulation: Transsynaptic and Intracellular Mechanisms David C. Klein, Ph.D.
Z01 HD 00704-05	Tetanus Toxin Effects and Localization in Neurons Elaine A. Neale, Ph.D.
ZO1 HD 00705-08	Functional Organization of the Nerve Terminal James T. Russell, Ph.D.
Z01 HD 00707-05	Pharmacological Studies of Synaptic Transmission <u>In Vitro</u> Mark L. Mayer, Ph.D.
Z01 HD 00708-05	Morphologic Studies of Neuronal and Non-Neuronal Cells in CNS Cell Cultures Elaine A. Neale, Ph.D.
Z01 HD 00709-03	Prevention of Neuronal Deficits Associated with AIDS Douglas E. Brenneman, Ph.D.
ZO1 HD 00710-01	Molecular Genetics of Neuroenterations Andres Buonanno, Ph.D.
ZO1 HD 01202-02	Regulation of Expression and Function of Neuropeptides During Development Y. Peng Loh, Ph.D.

Laboratory of Developmental Neurobiology

Considerable expansion and consolidation has taken place within the Laboratory of Developmental Neurobiology (LDN) during FY '89. The program of Dr. Y. Peng Loh has rejoined the LDN as the Section on Cellular Neurobiology and Dr. Andres Buonanno's Unit on Molecular Neurobiology has occupied newly renovated laboratories. Work is nearing completion on space in Bldg. 36 which will house Dr. Loh's group, Dr. David Klein's Section on Neuroendocrinology and Dr. Douglas Brenneman's Unit on Neurochemistry. Further renovations in Bldg. 37 will enhance the programs of Drs. Neale, Mayer and Nelson. Productive biweekly laboratory meetings in which current work of the various Units in presented and discussed has facilitated interactions between the groups. Collaborations within the Laboratory are progressing well and we anticipate further integration of the efforts of this rather diverse Laboratory when all the groups are located in Bldgs. 36 and 37. The LDN has been an active originator of the NIH Neuroscience Seminar Series that featured an extremely well-received group of lectures by outside speakers given at the Hughes Foundation Convent facility.

Section on Neurobiology

The functional architecture of the nervous system is critically dependent on the 'sculpturing' effects of neural activity patterns imposed by structured stimulation from the environment during development. Considerable progress has been made by Nelson, Fields, Yu and Neale in the last year in analyzing the activity-dependent synapse stabilization and elimination involved in this sculpturing. In the three-compartmental tissue culture system used by these workers, a plasticity phenomenon has been demonstrated whereby chronic stimulation (for 4-5 days) of sensory afferents to spinal cord (SC) neurons confers a synaptic advantage on the stimulated afferents relative to unstimulated afferents converging onto the same SC neurons. We have shown that specific blockade of one type of excitatory synaptic receptor, the NMDA receptor, on the SC neurons by APV prevents this response to chronic stimulation. The plasticity, however, is not exclusively related to NMDA receptor activation, because we detect the plasticity under conditions of NMDA receptor blockade if we raise the concentration of calcium ions in the culture medium. We hypothesize that free intracellular calcium ion concentration is critical in regulating both synapse-augmenting and synapse-eliminating cell biologic processes. The NMDA receptor is only one mechanism, albeit a potent one, involved in activity-dependent changes in intracellular calcium. Collaboration has begun with Dr. Russell in the Unit on Neuronal Secretory Systems with the aim of making direct measurement of this latter critical variable in living neurons and glial cells. Pharmacological manipulations to test hypotheses as to second messenger involvement in synapse development and effects of protease inhibitors on synapse elimination are contemplated.

Although ongoing electrical activity appeared to exert a positive effect on synapse stabilization, a detailed analysis of chronic phasic stimulation and neurite outgrowth indicated very little effect. However, the same stimulation induced an acute inhibition of neurite growth and a change in the morphology of the growth cone toward a more primitive form. The morphological substrate for the growth cone alteration, as well as the role of calcium in producing these acute changes and in the

eventual recovery, will be investigated. It remains possible that electrical activity, at a critical interval during synaptogenesis, may act to stabilize extant connections and inhibit further growth of neurites that have not yet established a synapse.

Unit on Neurochemistry

The major focus of the Unit on Neurochemistry is cellular communication which regulates epigenetic development of the central nervous system. The molecular basis of such communication is undoubtedly complex, but this group has pioneered the idea that neuropeptides mediate communication between neurons and nonneuronal cells. The results of these interactions have profound consequences for the shaping and composition of neuronal networks in the CNS. Progress in characterizing the significance of one peptide (vasoactive intestinal peptide) (VIP) in particular has been hampered by the lack of specific drug antagonists which could be used to delineate function both in vitro and in vivo. A hybrid peptide drug has been synthesized and found to have potent VIP antagonistic properties in such diverse assays as: sexual activity in rats, VIP-stimulated adenylate cyclase, VIP-mediated protein secretion from astroglia, VIP radioligand binding and neuronal survival in developing CNS cultures. In addition to providing much needed pharmacological agents for the study of neuropeptide function, this group has also studied the ontogeny of mRNA for various neuropeptides found in CNS cultures. Of particular importance was the discovery that large amounts of mRNA for VIP were present during a developmental period when rapid neuronal growth and differentiation occurs. The coincidence of the message for VIP and their previous studies of the neurotrophic aspects of exogenous VIP combines to make a strong case for the biological significance of this peptide during development. The complexity of the consequences of VIP's interaction with nonneuronal cells has begun to emerge in their studies of secreted proteins elicited by sub-nanomolar concentrations of the peptide. It is now apparent that VIP releases many proteins from cortical astrocytes, some of which increase the survival of developing neurons. The characterization of these glial-derived proteins is a major focus of future work for this group.

In parallel but complementary studies, this Unit continues to investigate the possibility that the hippocampal neuronal death produced by gp120 (the external envelope protein of the Human Immunodeficiency Virus) is caused by interference with endogenous neurotrophic substances, perhaps VIP. Recent work has suggested that gp120 does not act directly on hippocampal neurons, rather it interacts with nonneuronal cells to produce neuronal cell death. In addition, a neuronal survival assay has been used to detect neurotoxic material present in the cerebrospinal fluid from patients with AIDS dementia. All the toxicity associated with the CSF of these patients has been shown to be attenuated buy the drug, Peptide T, and by monoclonal antibodies to murine CD4, thus suggesting the specificity and possible prevention of these deleterious effects. In summary, this group is committed to the further exploration of neuropeptide-related mechanisms important to neurodevelopment and the application of these findings to human disease.

Unit on Cell Biology

Tetanus toxin has been studied in this Unit for several years, both in terms of its physiological effect on neurons and because of its usefulness as a marker for the neuronal surface membrane. These studies have been re-initiated, coincident with other developments in the Laboratory. The patch clamp technique allows the recording of electrophysiological events in very small cells, and thus it has become

possible to follow the electrical development of newly cultured neurons. We have demonstrated that tetanus toxin binds to the surface of such young neurons and can be used with immunohistochemistry to stain neurons in living (i.e., non-fixed) cultures. We have demonstrated further that Fragment C, the binding portion of the toxin molecule, labels young neurons equally well with the added advantage that it doesn't appear to interfere with electrophysiology. These results, together with our acquisition of a high sensitivity video camera and optical memory disk recorder, offer the possibility of following the morphological development of freshly plated cells. These studies, coupled with image analysis, would provide information on neuronal complexity and on events leading to the formation of contacts with target cells. The ability to obtain correlative electrophysiological analysis provides a functional component to the study.

The use of Fragment C as a neuronal stain, however, opens questions about the kinetics of its binding to neurons. Other cell biological issues arise concerning differences in the cellular processing of holotoxin and its binding and active fragments, and differences in the processing that occurs in young as opposed to more developed neurons. Preliminary studies have indicated that developmental state is related to the severity of neurotoxicity, similar to findings for tetrodotoxin. Central to all of these issues is the mechanism whereby the toxin blocks synaptic activity. Using radiolabeled toxin, Fragment C, and various characterized monoclonal antibodies, we have obtained data on some of the kinetic issues. Bound Fragment C can dissociate from the neuronal surface and retain its ability to re-bind; tetanus toxin's association with the neuron is more stable, and it is internalized more readily. A specific monoclonal antibody stabilizes the association of Fragment C, and has very little effect on holotoxin. Gel electrophoresis has provided a time course of toxin breakdown and indicates that the toxin is degraded in or on neurons and that breakdown products are released into the medium. Using these biochemical data, we will proceed to undertake morphologic studies involving immunoelectron microscopy. The fine structural localization of toxin will be correlated with its electrophysiological effects and may help in understanding how the toxin acts at the synapse and how some neurons recover function.

Unit on Molecular Neurobiology

A major form of interneural communication consists in the release by presynaptic neurons of neurotransmitters that diffuse to postsynaptic cells baring the receptors. The effects of the neurotransmitters on the postsynaptic cell vary according to the type of receptors expressed. The two major types of receptors located on postsynaptic targets are either linked to G proteins or activated directly by binding of the ligand. The latter, known a ligand-gated channels, open an ion selective pore when bound to a specific neurotransmitter. Regulating the temporal expression, location, and availability of functional receptors on the cell surface can modulate interneural communication, which could consequently affect fundamental processes such as cognition, learning and memory. A major interest of the Unit on Molecular Neurobiology is to study how the expression and function of ligand-gated channels are modulated during development and by neural activity. We have focused our studies on two types of ligand-gated channels; the nicotinic acetylcholine receptors (nAChRs) and the excitatory amino acid receptors (EAARs).

Although different ligand-gated ion channels bind different neurotransmitters and are selectively permeable to ions of different charge and radius, it was recently

discovered that they are evolutionarily related. The subunits of nAChRs, GABA and glycine receptors, which are members of this super gene family, have conserved four putative transmembrane spanning regions thought to line the ion pore, and an extracellular domain flanked by disulfide-bonded cysteines thought to form part of the agonist binding site. It is thought that EAARs are also members of the ligand-gated channel super gene family. Therefore, Drs. Raluca Eftemie and Dr. Andres Buonanno used degenerate oligonucleotide probes, coding for conserved sequences extending the intracysteine domain, to screen cDNA libraries for EAAR clones. In theory, the degenerate oligonucleotide mixture should hybridize to all members of the super gene family. To maximize the chances of identifying clones coding for EAARs, lobster muscle and Drosophila cDNA libraries were screened (invertebrates use glutaminergic transmission at their neuromuscular synapse, thus invertebrate muscle represents an enriched source of EAAR mRNA). A 1.7 kb Drosophila cDNA clone was partially sequenced and shown to have homology to members of the ligand-gated ion channel family in the intracysteine domain; other conserved sequences in the transmembrane domain have not been identified thus far. Twenty five genomic clones hybridized to the probe, these are presently being subcloned, sequenced, and characterized.

A Xenopus oocyte expression system was established in the laboratory by Dr. Buonanno to functionally identify the type of receptors coded by the cDNAs cloned. This technique consists in injecting in vitro synthesized RNA made from the cDNA clones into oocytes, and using two-electrode voltage clamp to access for neurotransmitter-induced currents. The system was initially optimized by injecting rat brain poly-A RNA and inducing depolarizing currents by the application of glutamate, kainate and quisqualate (glutamic acid analogs). The responses were qualitatively and quantitatively similar to those published by other groups. We have begun to test for transmitter-induced currents, oocytes injected with RNA synthesized from large pools of cDNA clones that hybridized to degenerate oligonucleotides coding for the intracysteine domain. The first batch of fifty lobster muscle cDNA clones failed to elicit responses when glutamate, quisqualate or kainate were applied to the bath. We plan to continue this approach, which is a modification of the "sib selection" procedure initially used to clone serotonin and substance K receptors, to screen lobster muscle and rat brain cDNA libraries for kainate and quisqualate receptors.

The neuromuscular junction historically has served as a model for understanding synaptogenesis and synaptic function. In mammals, synaptic transmission at the neuromuscular junction is cholinergic, and mediated by nAChRs. Although this ligand-gated channel has been studied extensively at the biochemical level, little is known about the mechanisms that regulate its transcription during development and by neurally-induced electrical activity. In collaboration with Drs. John Merlie and Michael Crowder (Washington University, St. Louis), Dr. Buonanno showed that a 750 bp upstream region of the mouse nAChR gamma subunit gene confers proper tissue-specific and developmental regulation when transfected into mouse C2 skeletal muscle cells. This work is being extended by Dr. Laura Lautens, who is delineating the enhancer sequences by : creating serial deletions in the 750 bp upstream sequence using the polymerase chain reaction procedure, inserting the constructs in chloramphenicol acetyltransferase expression vectors, and quantitating CAT activity in transfected muscle cells. In collaboration with Dr. Phil Nelson, we have started experiments to study the down-regulation of nAChR expression by electrical activity. Jonathan Smith is optimizing conditions to stimulate cultures of C2C12 skeletal muscle cells by applying field currents, and testing for the effect of these currents on nAChR gene transcription. Utilizing this approach, we plan to use constructs

containing transcription regulatory elements in CAT expression vectors to identify the cis-acting sequences that respond to electrical activity.

Unit on Neurophysiology and Biophysics

The Unit on Neurophysiology and Biophysics led by Dr. Mark Mayer has continued to explore the physiology, biophysics and cell biology of excitatory amino acid receptors. Experiments by Drs. Vyklicky, Patneau and Mayer use a fast perfusion system to rapidly applying excitatory amino acids. This is allowing analysis of dose response curves for activation of N-methyl-D-aspartate receptors on hippocampal neurons by transmitter candidates, such as L-glutamate and L-homocysteate, as well as providing valuable insight into structure activity relationships for agonist action at NMDA and non-NMDA receptors. L-glutamate is the most potent NMDA receptor agonist found in the brain (Kd = 2.2 μM), followed by L-homocysteate (12 μM) and L-aspartate (17 μM). Similar experiments are in progress with a family of sulfur amino acids, errors in the metabolism of which lead to severe neurological deficits in man. Due to the fast onset of desensitization and the neurotoxic action of NMDA receptor agonists, such experiments are difficult to perform and interpret without use of the fast perfusion system. The high potency of endogenous NMDA receptor agonists raise fundamental questions about the mechanisms which regulate the concentration of these neurotoxic transmitter substances in the extraneuronal environment.

Similar experiments by Vyklicky, Benveniste and Mayer are under way to measure the mechanisms by which glycine regulates desensitization at NMDA receptors and are leading to a kinetic model for NMDA receptor function. Major findings include an increase in the rate of onset of NMDA-evoked desensitization at constant glycine concentration, with similar kinetics to relaxations evoked by glycine concentration jumps at a constant dose of NMDA. One model which would account for this would be an absolute requirement for binding of both NMDA and glycine for ion channel activation, followed by an agonist triggered conformational change to a state of lower affinity for glycine, such that desensitization is induced by dissociation of glycine from the receptor channel complex. A number of experiments are in progress to test this.

Experiments by Benveniste, Mienville, Sernagor and Mayer have used concentration jumps with antagonists to explore structural features required for block of NMDA receptor activity by ligands which act competitively at the NMDA and glycine binding sites. The most interesting finding to date concerns the NMDA antagonists, CPP and AP5, which have similar equilibrium affinities, but markedly different association and dissociation rates. CPP is a conformationally restricted agonist based on a piperazine ring, and is similar in structure to AP7 in a folded conformation. Future experiments with other conformationally restricted antagonists are planned as these compounds are developed by the pharmaceutical industry, and could provide molecular insight into features required for activity as NMDA antagonists.

Section on Neuroendocrinology

The Section, directed by David C. Klein, has made a series of important discoveries of basic importance in signal transduction and molecular biology of the pineal gland. These advances are of importance to the pineal gland, which functions as part of a

system which measures the duration of the night period and converts this measure into a hormonal signal, the duration of elevated levels of melatonin at night. In addition, these findings have significance in the field of transsynaptic regulation of neuronal metabolism in general.

The advances made this past year which cover signal transduction will be reviewed first. Olga Nikodijevic discovered that there are receptors on the pinealocyte for adenosine which regulate cyclic AMP and cyclic GMP production. It was also found that adenosine is formed from extracellular ATP by an enzyme system which appears to be located on the external surface of pineal cells. This is important in signal transduction because ATP is released along with norepinephrine and other transmitters which are stored with ATP in a complex. Accordingly, these findings point to adenosine as a neural transmitter in the pineal gland.

Li Yu investigated phosphorylation of pineal proteins, with a special interest in identifying substrates of cyclic GMP dependent protein kinase. To accomplish this, he purified this enzyme from brain and found that incubation of cyclic GMP, purified cyclic GMP dependent protein kinase and pineal supernatant fractions results in phosphorylation of two proteins. This is the first clear evidence of any effect of cyclic GMP on pineal metabolism.

Juan Antonio Reig and Li Yu have also studied substrates of cyclic AMP dependent protein kinase in the pineal gland. This effort has revealed the presence of a 33 kDa phosphoprotein which is phosphorylated in intact cells by an adrenergic - cyclic AMP dependent mechanism. This protein is of further interest in signal transduction because it appears to exist in a complex with the $\beta\gamma$ subunit of GTP binding regulatory protein. A similar situation also exists in the retina, making these tissues striking exceptions to the generalization that the $\beta\gamma$ subunit is limited to the membrane. Hence, it is now clear that this regulatory system probably has cytoplasmic function. This team has also identified the αi subunit of the inhibitory GTP binding regulatory protein in pineal cytoplasm. It seems that these elements participate in a system which modifies signal transduction.

Anthony Ho and Connie Chik have discovered that norepinephrine increases the intracellular pH of the pinealocyte, and that this is accomplished by activation of an α_1-adrenergic $\rightarrow$ $[Ca^{2+}]i \rightarrow$ protein kinase C mechanism. This change in pH may be of special importance in the regulation of the cyclic GMP response to norepinephrine, because when this change is blocked the cyclic GMP response is markedly reduced. Another possible effect of pH may involve protein:protein interactions. It is known that the 33 kDa/$\beta\gamma$ complex described above dissociates at elevated pH.

Important advances in molecular biology of the pineal gland have been made by the Section in both signal transduction proteins and in melatonin forming enzymes. Melatonin is formed from serotonin by two enzymes. The first is N-acetyltransferase; Joan Weller isolated putative N-acetyltransferase clones using antiserum against sheep N-acetyltransferase raised in a collaborative effort with Georgetown University Staff. These clones have been sequenced by Patrick Roseboom. These clones will be further characterized. The second enzyme in melatonin synthesis from serotonin is hydroxyindole-O-methlyltransferase; Helena Illnerova cloned the human hydroxyindole-O-methyltransferase gene, which has now been sequenced by Susan Donohue. Future plans involve identification of the regulatory elements which cause the expression of this gene in the pineal gland and retina. Serotonin is formed from tryptophan in the pineal gland by two enzymes, the first of which has been studied by the Section. Joan Weller has cloned sheep

tryptophan hydroxylase, which has been sequenced in part in a collaborative effort with NIMH staff. cDNA probes are being used to study the photoneural regulation of the expression of mRNA coding for tryptophan hydroxylase. Finally, in a collaborative effort involving several laboratory members and members of the NEI, rat pineal S-antigen has been cloned and sequenced. This protein is found in pineal and retina only, and appears to be involved in signal transduction.

Unit on Neuronal Secretory Systems

The nerve terminal is a highly specialized region of a neuron, separated from the neuronal soma by an axon, whose function is to release neurotransmitter quanta and to regulate the number of quanta secreted. Secretion of neurotransmitters and other biologically active substances from nerve terminals forms the fundamental means by which the central nervous system (CNS) operates from the time of development to higher order functions in the adult. Modulation of the quantity of the transmitter released at the terminal may form the basis for all central nervous system functions, including integration of information, and long term information storage and retrieval. This modulation is achieved by transduction of information content in the action potential train, and by local influences at the nerve terminal via activation of receptors at the terminal and the resultant modification of the responses of the terminal membrane. Because of the complexity (cellular heterogeneity, and their complex organization), and extremely small size, basic understanding of the molecular mechanisms of nerve terminal function in the central nervous system is lacking. The program of the Unit on Neuronal Secretory Systems is focused on studying the biochemistry and physiology of the nerve terminal using the neurohypophysial neuroendocrine cells as the model system. The nerve terminals of the neurons of the hypothalamo-neurohypophysial system, which secrete vasopressin or oxytocin are discretely localized in the neurohypophysis, where they are accessible to experimental manipulations both in vivo and in vitro. These nerve terminals can be isolated from the neurohypophyses without contamination by the postsynaptic membrane, unlike nerve terminals from other regions in the central nervous system. Furthermore, in the posterior pituitary nerve terminals from only two very discrete types of magnocellular neurons are concentrated.

Studies on the elucidation of the functional organization of the nerve terminal form the central theme of this Unit. The current focus of the Unit is on the investigation of the importance of ionic channels and receptors on the initiation and modulation of secretion at the nerve terminal. Three major approaches are being employed: (1) Studies on the kinetics and magnitude of secretion from isolated nerve endings (neurosecretosomes). Agonists and antagonists of ion channels, receptors and enzymes are employed to study both the mechanism and regulation of secretion using this model. (2) Use of toxins and other biochemical studies to investigate the molecular mechanisms of calcium-induced exocytosis. (3) Quantitation of intracellular messengers (ion concentration measurements, and measurement of second messengers like cyclic GMP) involved in mediation, and modulation of secretion.

The neurosecretosome preparation (isolated neuroendocrine nerve endings) has been maintained in culture for over four days. During this time these nerve endings respond to depolarizing stimuli with calcium-dependent secretion of both vasopressin and oxytocin. They also respond to activation of receptors by modulation of hormone secretion. These cultured nerve endings are being used to study the dynamics of hormone secretion, its modulation by receptor occupation, and to identify ionic

channels on nerve terminals, using state-of-the-art biophysical techniques. Thus the channels and their modulation by neuropeptide receptors on the nerve terminals could be investigated.

The neurosecretosome preparation was used to study the kinetics of secretion in response to depolarizing stimuli with very high temporal resolution. These studies revealed that during prolonged depolarizations, secretion undergoes inactivation. Dr. Kemal Payza has found that this inactivation is dependent on extracellular calcium ions and not caused by the membrane potential change alone. Exposure to calcium ionophores, which cause secretion due to elevation of intracellular calcium in the absence of membrane depolarization, also causes partial inactivation of secretion. Dr. Payza has shown that calcium entry by itself is a necessary and sufficient stimulus for activation of secretion. Calcium is also necessary for the inactivation of secretion. Membrane depolarization per se does not seem to play a role in activation or inactivation of secretion other than to open voltage gated calcium channels which allows for calcium entry into the terminals. He has used the nerve terminal preparation to identify intracellular second messengers involved in the modulation of secretion. The effects of kappa opiates, somatostatin, and FMRF-NH2 on secretion, and their receptor-effector coupling are being investigated.

The neurosecretosome preparation provides an ideal model to study intracellular reactions involved in triggering and regulation of neurosecretion. The use of toxins that block secretion has been shown to provide a means of identifying cellular substrates important in the exocytosis machinery. In collaboration with Dr. Jane Halpern (CBER,FDA), we have shown that tetanus toxin at nanomolar concentrations completely blocks secretion induced by either depolarization of calcium ionophore. This inhibition is dependent on toxin internalization, and is blocked by tetanus antitoxin. We are attempting to identify the intracellular substrate of the toxin responsible for blockade of exocytosis.

The high resolution video imaging microscope adds a new dimension in the investigation of the functional organization of the nerve terminal. These studies indicate that this instrument is valuable in resolving long standing questions on the kinetics of calcium concentration increase in the terminal and its homeostasis. Preliminary experiments show that calcium entry into the neurosecretosomes mediated by depolarization occurs mainly at one pole of the nerve endings upon onset of the stimulus. Experiments are underway to correlate calcium, and H^+ ion transients with the rate of secretion. Furthermore, the mechanism by which opiate k-receptor activation brings about inhibition of secretion, the mechanism of tetanus toxin action, and the protein kinase-c mediated increase in secretion will be studied in terms of the involvement of the intracellular calcium concentration. Thus the quantitative light microscopy will form a central tool to quantitatively study intracellular signals using fluorescent probes.

Section on Cellular Neurobiology

The Section on Cellular Neurobiology under the direction of Y. Peng Loh studies the enzymology and regulation of biosynthesis, packaging, secretion and function of the ACTH/endorphin α-MSH family of neuropeptides. This family of peptides is involved in intercellular neurocommunication and neural development. The ACTH, α-MSH and endorphin peptides are synthesized in the brain and pituitary from a common, glycoprotein prohormone (pro-opiomelanocortin, POMC) of about 32,000 daltons in size. This group has continued to purify and characterize enzymes

involved in the proteolytic processing of POMC. A prohormone converting enzyme (PCE) has been purified from bovine intermediate and neural lobe secretory vesicles and characterized it as an acidic, aspartic protease which cleaves POMC at specific paired basic residues to yield ACTH, β-endorphin and Lys-γ3 MSH. PCE has been shown to be co-secreted with the POMC-derived peptides in a regulated manner from bovine intermediate lobe, confirming the presence of the enzyme in secretory vesicles. Dr. Nigel Birch has recently shown that PCE is a calcium-activated protease and that maximum stimulation of the enzyme was achieved with calcium concentrations in the 10-15 mM range. Such a range of free calcium concentrations has been reported in pituitary secretory vesicles. Furthermore, it was shown that the extent of POMC processing was dependent on calcium concentration. Thus, differences in the intravesicular free calcium concentration can potentially determine the different degree of formation of some of the smaller POMC cleavage products (eg. β-endorphin, joining peptide) in the intermediate and anterior lobes of the pituitary.

Dr. Birch has also explored the possibility that 'O'glycosylation of N-POMC may regulate the differential processing of this peptide in the two lobes of the pituitary. He incubated 'O'glycosylated N-POMC found primarily in anterior lobe and the non-'O'glycosylated N-POMC from the intermediate lobe with PCE and showed that the non-'O'-glycosylated form was cleaved to yield γ3MSH 7-8 times faster than the 'O'glycosylated form. The close proximity of the 'O'glycosylated group to the cleavage site may have resulted in steric hindrance to the enzyme preventing efficient cleavage. His findings are the first demonstration that 'O'glycosylation near a cleavage site can regulate the processing of a pro-protein and can account for the processing of N-POMC to γ3MSH only in the intermediate lobe of the pituitary.

Dr. Loh has continued to investigate the signals involved in targeting POMC to the regulated pathway. Fusion genes of different POMC cDNAs which had varying lengths of the 3' end deleted were fused to the CAT gene. Each of these constructs were expressed in AtT-20 cells using the vaccinia virus expression system. The CAT/POMC fusion proteins were shown to be expressed by AtT-20 cells using the immunofluorescence method of staining for CAT. Immunofluorescence studies revealed that cells expressing CAT fusion proteins containing $\geq$25 amino acids of the N-terminal POMC sequence were sufficient to route the prohormone into secretory vesicles. She also showed that forskolin treatment stimulated the secretion of CAT activity into the medium from stable transformants expressing CAT/POMC fusion protein with $\geq$25 amino acids of N-POMC. This regulated secretion of CAT activity confirmed the targeting of fusion proteins with >25 amino acids of N-POMC into secretory vesicles. Dr. Morgens Fenger has made several POMC/CAT constructs with mutations at some of the amino acids and expressed them in AtT-20 cells. This line of research should reveal the critical amino acids necessary to preserve the targeting signal of POMC.

In order to understand the role of POMC-derived peptides in neural development, the temporal/spatial expression of POMC was examined by <u>in situ</u> hybridization and immunocytochemistry. The first appearance of both POMC mRNA and immunoreactive POMC/ACTH was observed in the presumptive arcuate nucleus of embryonic brain on gestational day 10-1/2 (E10-1/2). Immunostained fibers were also evident on E10-1/2 in the lateral and dorsal diencephalon. POMC expression cells in the anterior and intermediate lobes of the pituitary gland first appeared on E12-1/2 and E14-1/2, respectively. These findings reveal for the first time that POMC is expressed very early in development and, interestingly, is expressed in the CNS before the pituitary. Dr. Adrian Rius showed that the ACTH immunoreactivity

seen at E10-1/2 was due to intact POMC and processing of the prohormone did not begin until E11-1/2. HPLC analysis of processed products at E11-1/2 revealed that des-acetyl α-MSH and β-endorphin were the major POMC-derived peptides present. No α-MSH was observed. In collaboration with Dr. C. Coscia (St. Louis University), Dr. Rius has shown that μ receptors first appear in mouse embryos at E12-1/2, one day after POMC processing in the CNS has begun. The embryonic μ receptor exhibited the same Kd as in adult brain, but the maximum specific binding was different. Moreover, displacement studies with various ligands indicate that these early embryonic μ receptors were specific for the POMC-derived peptide, β-endorphin. It appears, therefore, that as early as E12-1/2, the embryonic pattern of processing of POMC is already established and the receptor for at least one of these POMC-derived peptides (β-endorphin) is present, indicating that the POMC system is fully functional at this stage to exert its effect on CNS development. The role of des-acetyl α-MSH and β-endorphin in neuronal cell proliferation and neurite outgrowth is currently being investigated.

In order to address the issue of how the initiation of neuropeptide phenotypes are regulated in the vertebrate CNS, Dr. Hayes has continued to use in situ hybridization histochemistry to determine the temporal map of POMC mRNA expression in the embryonic frog CNS. POMC gene expression was first seen at developmental day 1.3 (Stage 28) in cells of the embryonic stomodeal-hypophysial plate. By day 2.3 (Stage 37/38), they near the notochord, where they are presumed to form the anterior and intermediate lobes of the pituitary. It appears from these in situ studies that many pituitary precursor cells are already differentiated as they pass in proximity to the developing brain. In the CNS, POMC mRNA labeling was first seen at Stage 31 in the neural tube in cells of the diencephalon. These cells appeared to radiate from the point at which the hypopyseal plate made its closest pass with the embryonic brain, raising the hypothesis that the onset of expression of this gene in brain is dependent on interactions with the hypophyseal cells.

To understand the regulation of expression of the POMC and pro-enkephalin genes during CNS development, Drs. May Wong and Judith Hewitt have cloned these two genes in the Xenopus laevis. Dr. Wong has now obtained 8 kb of 5' DNA sequences of the pro-enkephalin gene and is currently identifying the putative regulatory sequences.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00047-20 LDN

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Biochemical Studies of Neurons and Other Cell Types

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Brenneman	Pharmacologist	LDN,NICHD
Others:	R. Alderson	Staff Fellow	LDN,NICHD
	D. Warren	Bio. Lab. Tech.	LDN,NICHD
	S. McCune	NRSA Fellow	LDN,NICHD

COOPERATING UNITS *(if any)* Lab. of Cell Biol., NIMH (L. Eiden, D. Agoston); Lab. Mol. Genetics, NICHD (I. Gozes); Clinical Neurosci. Br., NIMH (S. Paul, H. Sheng, M. Schultzberg); Dept. Physiol., University College, Cardiff (G. Foster); Dept. of Physiology, Univ. of Leicester (I. Forsythe); Dept. of Organic Chem., Weizmann Institute (M. Fridkin).

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Unit on Neurochemistry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	1.9	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The influence of electrical activity on neuropeptide gene expression was studied with cell cultures derived from fetal mammalian central nervous system. Electrical blockade was shown to markedly reduce mRNA for methionine-enkephalin during specific periods of development. Removal of electrical blockade resulted in restoration of neuropeptide message and peptide immunoreactivity. In contrast, mRNA for vasoactive intestinal peptide (VIP) was influenced by electrical activity to a lesser extent.

A novel VIP antagonist was devised. Pharmacological activity of the antagonist was demonstrated by the following: inhibition of VIP-stimulated cAMP accumulation in cortical astrocytes, VIP radioligand binding studies in astrocytes, neuronal cell death in spinal cord cultures and inhibition of sexual activity in rats with reduced masculine potential. In addition, two-dimensional PAGE analysis of secreted astrocyte proteins revealed that 15 of 333 spots were specifically increased by VIP.

Antiserum to interleukin-1 decreased neuronal survival in developing spinal cord cultures. Vasoactive intestinal peptide, a substance of demonstrated neurotrophic activity, prevented the cell death associated with anti-interleukin-1.

A rat alpha-2 adrenergic receptor was cloned and characterized. The receptor consists of 1374 bp and would encode a protein of 458 amino acids. The sequence is 90% homologous to the human kidney alpha-2 receptor. By Northern analysis, the rat genomic clone is expressed in embryonic day 15 rat and to a greater degree in adults. In addition, a 1.8 kb message that cross-hybridized with the alpha-2 probe was found to be expressed only in embryos and not adults.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HD 00056-14 LDN

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biosynthesis, Processing & Secretion of Neuropeptides & Pituitary Peptide Hormones

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Y. Peng Loh	Section Chief	LDN, NICHD
Others:	Nigel Birch	Visiting Fellow	LDN, NICHD
	Mogens Fenger	Visiting Fellow	LDN, NICHD
	Toshiyuki Chikuma	Visiting Fellow	LDN, NICHD
	Anders Bjartell	Guest Researcher	LDN, NICHD
	Howard Tracer	Medical Staff Fellow	LDN, NICHD
	Winnie Tam	Microbiologist	LDN, NICHD
	Osvaldo Gigliotti	Stay-in-school Stud.	LDN, NICHD

COOPERATING UNITS *(if any)*

Lab. of Cell Biology, NIMH (M. Brownstein); Lab. of Viral Diseases, NIAID (B. Moss & T. Fuerst); Dept. of Psych., Lund Univ. Sweden (R. Ekman); IMBICE, La Plata, Argentina (F. Estivariz)

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Section on Cellular Neurobiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.41	2.83	1.58

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

Pituitary secretory vesicle enzymes involved in the processing of pro-opiomelanocortin (POMC, pro-ACTH/endorphin) and pro-vasopressin were studied. A 70,000 mol. wt. paired basic residue specific prohormone converting enzyme (PCE), previously purified and characterized as an aspartic protease, has been shown to be structurally related to Cathepsin D. PCE was shown to be secreted from bovine intermediate lobe together with alpha-MSH, in a co-ordinately regulated manner. Pepstatin A, an inhibitor of PCE, blocked processing of POMC in the mouse intermediate lobe further supporting a physiological role of the enzyme in vivo. Cloning of this enzyme is in progress. Studies were conducted to uncover mechanisms underlying the differential processing of POMC in the anterior and intermediate pituitary and brain. In vitro experiments show that calcium can regulate the extent of cleavage of POMC to smaller products, through the activation of PCE. Thus, the regulation of intravesicular calcium concentration may play a role in determining the quantitative differences in processing of beta-LPH to beta-endorphin in the anterior versus the intermediate lobe. Another finding was that 'O'glycosylation at Thr45 of N-POMC inhibited PCE from cleaving the Arg-Lys50 bond to yield N-POMC1-49 and Lys50gamma3MSH. Since the non-'O'glycosylated form of N-POMC is found in intermediate but not anterior lobe, this post-translational modification of POMC may account for the presence of N-POMC1-49 and Lys50gamma3MSH only in intermediate lobe. The signal involved in the targeting of POMC into the regulated secretory pathway was also investigated. Truncated POMC/CAT constructs were transfected into the endocrine AtT-20 cells and the intracellular localization of the expressed fusion protein analyzed by immunofluorescence histochemistry using anti-CAT. Furthermore, stimulated secretion studies with forskolin were done on stable transformants of AtT-20 cells transfected with various POMC/CAT constructs to verify localization of fusion proteins in secretory vesicles. The morphological and secretion data suggest that the signal for targeting POMC into the regulated pathway resides in the N-terminal 25 amino acids of the prohormone.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00064-13 LDN

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neurobiologic Studies of Neruons and Glia in Cell Culture

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.G. Nelson	Head	LDN, NICHD
Others:	C. Yu	Visiting Associate	LDN, NICHD
	E.A. Neale	Staff Fellow	LDN, NICHD
	D. Fields	IRTA Fellow	LDN, NICHD
	S. Fitzgerald	Biologist	LDN, NICHD

COOPERATING UNITS *(if any)*

Montefiore Med. Center, N.Y. (J. Moskal)

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Unit on Neurochemistry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dissociated cell cultures of fetal mouse spinal cord (SC) and dorsal root ganglion (DRG) neurons have been used in conjunction with a 3-compartment culture system to study the process of activity-dependent synapse augmentation and elimination.

With this system, two independent populations of DRG neurons converge on and synapse with a common population of SC neurons. Chronic (3-4 days) electrical stimulation of one set of DRG axons results in this population establishing stronger synaptic connections with the target SC neurons relative to the convergent non-stimulated DRG axons.

The NMDA receptor antagonist, APV, blocks this plasticity, but in solutions with raised calcium ion concentration (3 mM vs. 1.8 mM) plus AVP, stimulation again produces stronger synaptic connections from the stimulated axons as compared to those from the non-stimulated convergent axons. We infer a critical role for an increased intracellular calcium in the process of activity-dependent synapse competition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00094-19 LDN

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pineal Regulation: Environmental and Physiological Factors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.C. Klein	Head	LDN,NICHD
Other:	J.T. El-Hage	IRTA	LDN,NICHD

COOPERATING UNITS *(if any)*
Georgetown Univ. (M.A.A. Namboordiri)

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.6	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates the environmental and physiological regulation of the pineal gland, exclusive of transmembrane and intracellular regulatory mechanisms (see Z01 HD 00095-19 LDN). The pineal gland is part of the melatonin rhythm generating system, a neural circuit which includes a circadian clock in the suprachiasmatic nucleus (SCN); the SCN is reset and entrained by light acting through the eye. It has been proposed that the SCN pineal circuit passes through the paraventricular nucleus of the hypothalamus (PVN). Recent work was completed which supports this with the demonstration that electrical stimulation of PVN stimulated the production of melatonin at a near physiological rate. In other studies, the regulation of pineal phospholipase C has been studied; and the developmental appearance of sodium,potassium-ATPase has been examined. It has been discovered that sodium,potassium-ATPase develops after birth, as indicated by both ouabain binding and two indices of enzyme activity, ATP hydrolysis by membrane preparations and uptake of rubidium. Results indicate a high affinity form of sodium,potassium-ATPase, similar to the alpha-plus form which has been described in the brain, is the dominant form present in the pineal gland. This indicates that another mechanism might generate membrane potential before this time.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00095-19 LDN

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pineal Regulation: Transsynaptic and Intracellular Mechanisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.C. Klein	Head	LDN,NICHD
Others:	A.K. Ho	Visiting Fellow	LDN,NICHD
	J. Weller	Chemist	LDN,NICHD
	C. Chik	Guest Researcher	LDN,NICHD
	J.A. Reig	Guest Researcher	LDN,NICHD
	O. Nikodijevic	Guest Researcher	LDN,NICHD
	S. Donohue	Biotechnology Fellow	LDN,NICHD
	P. Roseboom	Biotechnology Fellow	LDN,NICHD
	Y. Li	Visiting Fellow	LDN,NICHD

COOPERATING UNITS *(if any)*

NCI (W. Anderson, T.P. Thomas); Georgetown U. (M.A.A. Namboodiri); NEI (T. Shinohara); AFRI (J. Halperin); Yale U. (R. Levenson)

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neuroendocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.3	5.2	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to discover the molecular basis of neurochemical transduction mechanisms, using the pineal gland as a model. Efforts are directed at determining the details of the chemical and ionic components of transmembrane signalling processing and in the neural regulation of gene expression. The most important advances made in the first area were those that have clearly indicated that cAMP and cGMP are regulated by a two receptor mechanism which appears to be focused on the regulation of adenylyl and guanylyl cyclases. One leg of this pathway activates these enzymes via GTP binding regulatory proteins, similar to GSalpha. This leg is controlled by beta-adrenergic or VIP receptors; activation of this leg produces only partial stimulation of cAMP and cGMP accumulation. Activation of the other leg is via alpha1-adrenergic receptors. This activates protein kinase C which acts, perhaps on the regulatory or catalytic proteins, to increase the activation of adenylyl and guanylyl cyclase. Activation of protein kinase C occurs as a result of an increase in [calcium]i and in diacylglycerol production by phospholipase C. In addition, in the regulation cGMP, there appears to be a strong requirement for activation of phospholipase A and for an increase in [calcium]i. In the area of the neural control of gene expression, advances have been made in purifying N-acetyltransferase and hydroxyindole-O-methyltransferase, and in isolating cDNA clones coding for these enzymes.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00704-05 LDN

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Tetanus Toxin Effects and Localization in Neurons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Elaine A. Neale	Head	LDN,NICHD
Others:	Holly I. Trenchard	IRTA Fellow	LDN,NICHD
	Linda M. Bowers	Biologist	LDN,NICHD
	Sandra C. Fitzgerald	Biologist	LDN,NICHD

COOPERATING UNITS *(if any)*

Div. of Bacterial Products, Center for Biologics Evaluation Research, Food and Drug Administration (W.H. Habig)

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Unit on Cell Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Fragment C (FrC), the binding portion of the tetanus toxin molecule, can be used in combination with a non-neutralizing monoclonal antibody, 18.2.12.6, as a neuronal label for the immunohistochemistry of neurons in primary cell culture. Staining and binding experiments in living cultures indicate that both ligands bind to neurons, although much of the FrC/18.2.12.6 can dissociate from and subsequently rebind to the neuronal membrane, whereas tetanus toxin/18.2.12.6 is internalized, degraded, and rapidly excreted.

FrC/18.2.12.6 is not toxic for developing neurons, although tetanus toxin, when applied to cultures at the time of synaptogenesis, causes a reduction in neuronal survival.

The elimination of toxin from neurons is non-linear at early times after binding, but stabilizes later with a half-time of about 6.5 days. The elimination of toxin/18.2.12.6 proceeds with similar kinetics, suggesting that the complex might be used for immunoelectron microscopic localization of intracellular toxin sites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00705-08 LDN

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Functional Organization of the Nerve Terminal

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Russell	Head	LDN,NICHD
Others:	K. Payza	IRTA Fellow	LDN,NICHD

COOPERATING UNITS *(if any)*

Lab. of Biophysics, NINCDS (E. Stanley, G. Ehrenstein); LMB, NIMH (D. Neville); CBER, FDA (J. Halpern); NBS (S. Krueger); INSERM Strasbourg, France (J. Nordmann); Inst. of Medical Physiology-C, Univ. of Copenhagen, Denmark (N. Thorn).

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Unit on Neuronal Secretory Systems

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	3.5	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The research program is directed towards studying the biochemistry and physiology of the nerve terminal. The neurohypophysial nerve terminals are used as the model to study the importance of ion channels and receptors on initiation and modulation of secretion. These include studies on secretion of vasopressin and oxytocin from isolated intact posterior pituitaries and isolated neurosecretosomes, and ion transients in nerve terminals. We have developed methods to maintain neurosecretosomes under tissue culture conditions and have used them to study the mechanism of secretion, its inactivation, and modulation. This preparation is suitable for high resolution microscopy and patch clamp analysis of ion channels. Neurosecretosomes respond to depolarizing stimuli with markedly elevated secretion. Calcium ionophores also cause secretion in sodium-free media in the absence of applied depolarization. Tetanus toxin in nM concentrations blocks secretion of both hormones induced by depolarizing stimuli, as well as by calcium ionophores. Biochemical and pharmacological approaches are being used to describe the mechanism of toxin induced blockade of exocytosis. Secretion of both hormones was inhibited by the opiate kappa-receptor agonists, dynorphin and U50488H. This receptor-mediated modulation of secretion at the nerve terminal is under investigation. Hormone release from neurosecretosomes was found to be inactivated rapidly under maintained depolarizations. This inactivation was not voltage-dependent, and could also be elicited by treatment with calcium ionophores. Secretion during prolonged stimulations depended on continued opening of voltage-dependent calcium channels, suggesting the relevant channels are not inactivated by membrane depolarization for up to 4 min with 25 mM potassium. A vertical optical bench microscope with digital image processing capabilities has been set up in order to quantitate intracellular ion transients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00707-05 LDN

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pharmacological Studies of Synaptic Transmission In Vitro

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.L. Mayer	Head	LDN,NICHD
Others:	J.M. Mienville	Guest Researcher	LDN,NICHD
	D. Patneau	IRTA Fellow	LDN,NICHD
	E. Sernagor	Visiting Fellow	LDN,NICHD
	L. Vyklicky	Visiting Fellow	LDN,NICHD
	S. Fitzgerald	Biologist	LDN,NICHD
	C. Winters	Chemist	LDN,NICHD

COOPERATING UNITS *(if any)*
Laboratory of Neurophysiology, NINDS (M. Benveniste and J. Clements); Section on Instrumentation, Research Services Branch, NIMH (B.M. Smith and S. S. Hsiao)

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Unit on Neurophysiology and Biophysics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.8	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This Unit investigates the mechanism of action of excitatory amino acids as synaptic transmitters and neuromodulators in the vertebrate CNS, utilizing cell culture and electrophysiological techniques in conjunction with a fast perfusion system for applying drugs and ions to nerve cells under voltage clamp. Dose response curve analysis for NMDA receptors shows the potency sequence decreases in the order L-glutamate, L-homocysteate, L-aspartate, L-homocysteinesulphinate, NMDA. For quisqualate receptors the sequence decrease is quisqualate, AMPA, L-glutamate, with L-aspartate devoid of activity. Modulation by glycine of responses at NMDA receptors has been studied using concentration jump experiments. Receptors at equilibrium are perturbed by rapid changes in the concentration of agonist (NMDA) or modulator (glycine). Similar experiments have been performed using fast application of a variety of competitive and noncompetitive NMDA antagonists, including AP5, CPP, 7Cl-Kynurenic acid, Zn and Mg. Responses to NMDA show strong desensitization at low (less than 300 nM) but not high (more than 1 microM) concentrations of glycine. Glycine antagonists promote desensitization. The competitive antagonists CPP and AP5 are of similar potency as NMDA antagonists at equilibrium, but the dissociation rate constant for CPP is 20 times slower than for AP5, suggesting that the conformationally restricted structure of CPP also slows the on rate for binding to NMDA receptors. Experiments with tricyclic antidepressants and phenothiazines suggest that these drugs act as NMDA antagonists by binding to the anaesthetic recognition site at which MK-801 and PCP also have activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00708-05 LDN

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Morphologic Studies of Neuronal and Non-neuronal Cells in CNS Cell Cultures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.:	Elaine A. Neale	Physiologist	LDN,NICHD
Others:	R. Douglas Fields	IRTA Fellow	LDN,NICHD
	Linda M. Bowers	Biologist	LDN,NICHD

COOPERATING UNITS *(if any)*

Lab. of Neurophysiology, NINDS, NIH (T.J. Smith, Jr.)

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Unit on Cell Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.6	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Action potentials in mammalian neurons initiate changes in growth cone morphology and arrest the outgrowth of neurites. A slow process of accommodation enables continued outgrowth in the presence of electrical stimulation. The effects of electrical activity are believed to be caused by changes in intracellular calcium levels. By affecting the motility of growth cones, electrical activity in neural circuits could influence the pattern of connections formed during development.

Image processing software has been used to develop a method for detecting the edges of a neuron and filling the interior of the continuous border. The resulting binary image of the neuron can be used in a computer-assisted analysis of its fractal dimension. The fractal dimension, as a descriptor of neuronal complexity, should prove useful in studies of neuronal development and of those phenomena which affect development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00709-03 LDN

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Prevention of Neuronal Deficits Associated with AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Brenneman	Pharmacologist	LDN, NICHD
Others:	S. Fitzgerald	Biologist	LDN, NICHD
	S. McCune	NRSA Fellow	LDN, NICHD
	G. Buzy	Guest Worker	LDN, NICHD

COOPERATING UNITS *(if any)*

Biological Psychiatry Branch, NIMH (C.Pert); Dept. Oncology, Long Island Jewish Hospital (F. Siegal); Head Trauma Unit, Walter Reed Army Hospital (A. Martin, A Salazar).

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Unit on Neurochemistry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	0.6	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dissociated hippocampal cultures from the fetal mammalian central nervous system were used to study interactions between the envelope protein (gp120) from the Human Immunodeficiency Virus and developing neurons. Previous studies indicated that purified gp120 from several HIV isolates potently produced neuronal cell death in a population of murine hippocampal neurons. We now report that a recombinant gp160 from the LAV encoding sequences also produced neuronal cell death, whereas recombinant gp160 expressed in insect cells had no effect on neuronal survival.

Cerebrospinal fluid, taken from AIDS patients at various stages, was tested for neurotoxicity in the hippocampal neuronal cell death assay. Of 18 sero-positive patients, 50% were found to contain significant (less than 20%) neuronal cell death at 1:100,000-fold dilution. In all cases of neuronal cell death induced by the dilute CSF, co-treatment with 10 nM peptide T in the assay increased neuronal survival significantly. One out of 10 CSF samples from patients with other neurodegenerative diseases showed significant neuronal cell death that was prevented by peptide T.

Previous studies indicated that monoclonal antibodies against murine CD4 prevented neuronal cell death associated with gp120. Similarly, a monoclonal antibody was also found to prevent hippocampal neuron death after treatment with dilute CSF from patients with AIDS dementia.

Hippocampal cultures that were comprised predominately of neurons were not vulnerable to neuronal cell death after treatment with gp120.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00710-01 LDN

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetics of Neurointeractions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Buonanno	Head	LDN,NICHD
Others:	R. Eftemie	IRTA	LDN,NICHD
	L. Lautens	Visiting Fellow	LDN,NICHD
	J. Smith	Chemist	LDN,NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Unit on Molecular Neurobiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	1.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Ligand-gated ion channels play a central role in interneural communication, they are the site of action of different psychoactive drugs, and have been implicated in simple forms of memory and learning. Our efforts have concentrated in identifying excitatory amino acid receptors by using conventional DNA recombinant techniques, in combination with a Xenopus oocyte expression system and two-electrode voltage clamp . Utilizing oligonucleotide probes coding for sequences conserved in members of the ligand-gated super gene family, we screened cDNA and genomic libraries of Drosophila and lobster. A Drosophila cDNA clone containing a short segment of sequence homologous to acetylcholine and GABA receptors was obtained; this clone is being characterized further. Approximately eight unique Drosophila genomic clones were obtained; four do not seem to code for neurotransmitter receptors and we are continuing to analyze the remaining clones. Screening of unamplified lobster cDNA libraries with highly degenerate oligonucleotide mixtures yielded approximately fifty weakly hybridizing clones. Oocytes injected with RNA synthesized in vitro from these lobster cDNA clones did not respond to the application of glutamate or its analogs.

We have recently started to characterize the nicotinic acetylcholine receptor gamma subunit enhancer by creating serial deletions, with the polymerase chain reaction procedure, in the upstream regulatory sequences of the gene. The enhancer activity will be assayed in C2C12 muscle cells transfected with the differentiated chloramphenicol acetyltransferase (CAT) constructs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01202-02 LDN

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Expression and Function of Neuropeptides During Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Y.P. Loh	Section Chief	LDN,NICHD
Others:	May Wong	Senior Staff Fellow	LDN,NICHD
	Adrian Rius	Visiting Fellow	LDN,NICHD
	William Hayes	NRC/Biotech. Assoc.	LDN,NICHD
	Judith Hewitt	NRC/Biotech. Assoc.	LDN,NICHD

COOPERATING UNITS *(if any)*

Dept. Biochemistry, St. Louis Univ. (C. Coscia)

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Section on Cellular Neurobiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Neuropeptides have been shown to have trophic and mitogenic effects, and have been implicated to play a role in development. The objectives of this project are to study the developmental regulation of expression of two neuropeptide genes coding for the pro-opiomelanocortin (POMC) and the pro-[met]enkephalin family of peptides, and to investigate the role of these peptides in development, particularly in the central nervous system (CNS). Two model vertebrate systems: the frog (Xenopus laevis) and mouse were used. Initial studies focused on defining the temporal and spatial expression of POMC and [met]enkephalin peptides during development. In situ hybridization histochemistry revealed the first appearance of POMC mRNA in the mouse CNS in the region of the presumptive arcuate nucleus, at embryonic day 10-1/2 (E10-1/2), and in the anterior lobe and intermediate lobe of the pituitary at E12-1/2 and E14-1/2 respectively. Immunocytochemical and biochemical analyses showed that the POMC was expressed at E10-1/2 but processing of the prohormone began only a day later, at E11-1/2. The major embryonic POMC-derived products were ACTH1-17, ACTH1-14, desacetyl alpha-MSH, ACTH1-39 and beta-endorphin, indicating that all the processing enzymes were present at this stage. In the frog, POMC was first seen in embryos at Stage 28 in the stomodeal-hypophyseal plates and later in the neural tube at Stage 31. The early expression of POMC in the developing embryo suggests that this family of peptides may be important in neurogenesis. The frog POMC and pro-enkephalin genes including the 5' upstream regulatory region have been cloned. Work is now in progress to identify the regulatory elements, a prerequisite to identifying the factors that trigger the activation of these genes during development.

LABORATORY OF DEVELOPMENTAL PHARMACOLOGY (LDP)

Z01 HD 00136-21	Molecular Pharmacogenetics Daniel W. Nebert, M.D.
ZO1 HD 00504-02	Cloning of the AH Receptor Gene Alvaro Puga, Ph.D.

Laboratory of Developmental Pharmacology

SUMMARY

The LABORATORY OF DEVELOPMENTAL PHARMACOLOGY studies the molecular biologic mechanisms of gene expression involving drug-metabolizing enzymes. The clinical discipline involving the study of genetic differences in drug metabolism has been termed **pharmacogenetics**. Cytochromes P450 are enzymes involved in the oxidative metabolism of steroids, fatty acids, prostaglandins, leukotrienes, biogenic amines, pheromones, and plant metabolites. These enzymes also metabolize innumerable chemicals in foodstuff, as well as drugs, chemical carcinogens and mutagens, and other environmental contaminants. The large degree of overlapping substrate specificities, classes of inducing agents, and drug-drug interactions have caused great difficulty in P450 studies at the level of catalytic activities and protein immunochemistry. P450 enzymes represent the classical "Phase I" metabolism in which the substrate is oxygenated. "Phase II" enzymes often use the oxygen as a site for further metabolism (e.g. quinone reduction glucuronidation, and sulfate, glutathione, or glycine conjugation). Detoxification usually requires both Phase I and Phase II enzymes. Ironically, the intermediate formed after Phase I metabolism is often more toxic, carcinogenic or mutagenic than the original Phase I substrate.

Hundreds of drugs and other chemicals are known to stimulate (induce) their own metabolism or the metabolism of structurally-related compounds. In addition, steroids, prostaglandins, and small peptide hormones have been found to regulate some of these activities. The mechanisms surrounding the induction of these enzymes and expression of these genes are of central importance to such fields as fundamental molecular genetics, developmental biology, teratogenesis, chemical carcinogenesis and mutagenesis, endocrinology, limology, drug addiction and tolerance, and toxicity. This laboratory presently comprises one Section.

A. The **Section on Pharmacogenetics**, under the direction of **Daniel W. Nebert, M.D.**, is interested in the regulation and expression of genes encoding Phase I and Phase II drug-metabolizing enzymes. Work in this Section is divided into four camps: P450 gene nomenclature and evolution, P450 gene regulation, oxidative stress, and clinical applications in the field of pharmacogenetics. Experimental systems include the use of recombinant DNA technology, inbred mouse strains, transgenic mice, somatic cell genetics and mutant lines in culture, and clinical material.

P450 gene nomenclature and evolution. Most of the Phase I enzymes represent P450 proteins. The P450 gene superfamily is presently known to comprise sixteen P450 gene families, nine of which exist in all mammals. The P450 gene superfamily is ancient and has expanded via divergent evolution. The ancestral P450 gene was present probably more than two and a half billion years ago. Estimates of the unit evolutionary period (UEP; millions of years required for 1% divergence in amino acid sequence) range between 4 and 14, indicating a striking nonlinearity for the P450 superfamily. Four major factors might contribute to this nonlinearity: (i) archeologic evidence suggests that evolution over eons of time might not be uniform; (ii) certain amino acids or regions of the P450 protein might need to be

retained, or conserved, in order for the enzyme to carry out the catalytic function needed for survival advantage to the organism; (iii) "molecular drive," a nonMendelian mechanism operationally distinct from natural selection and genetic drift, might aid the organism in spreading new variant genes in response to severe selective pressures such as dietary changes; and (iv) gene conversion events can reestablish 100% similarity in a portion (or all) of a gene that had been considerably diverged from its homologous neighbor located nearby on the same chromosome. In order to understand better the evolution of the P450 superfamily, this Laboratory has recently isolated and sequenced the first P450 gene from trout and from insect.

P450 gene regulation. At the present time, regulation of CYP1A1 gene expression has been more extensively characterized than that of all other P450 genes combined. This Laboratory has pioneered these regulatory studies through use of the mouse hepatoma Hepa-1 wild-type (wt) cultures and receptor-defective and Cyp1a1 metabolism-deficient mutant cell lines. These lines have been used for transfecting the reporter gene chlorampherical acetyltransferase (CAT gene) driven by various upstream regions of the Cyp1a1 gene. It can thus be determined which upstream regions require a functional aromatic hydrocabon (Ah) receptor and which regions require Cyp1a1 metabolism. Upstream Cyp1a1 regulatory elements include: (i) the TATA box; (ii) a proximal element between -245 and -150 that is absolutely essential for constitutive and inducible gene expression; (iii) a distal element, i.e. the tetrachlorodibenzo-p-dioxin (TCDD)-inducible enhancer between -1100 and -880; and (iv) and additional element that participates in a negative autoregulatory loop. Both the TCDD-inducible enhancer and the negative autoregulatory loop appear to require a functional aromatic hydrocarbon (Ah) receptor. The transcriptional preinitiation complex that up-regulates these genes is believed to include the Ah receptor (bound to endogenous or foreign ligand) and at least one other protein that confers chromatin binding properties. Site-directed mutagenesis of these elements, in vivo genomic footprinting, competition studies with gel mobility shift assays, and requirements for phosphorylation are underway for the CYP1A1 gene in mouse, human and trout, as well as for the mammalian CYP1A2 gene.

Oxidative stress. Metabolism of endogenous substrate(s) by the Cyp1a1 gene product not only appears to control its own constitutive expression but also the expression of at least five other genes encoding enzymes with coordinate metabolic functions--Cyp1a2, NAD(P)H:menadione oxidoreductase (Nmo-1), aldehyde dehydrogenase (Aldh-1), UDP glucuronosyltransferase (Ugt-1), and glutathione transferase (Gt-1). All six of these TCDD-inducible genes have been cloned, are under control of the Ah receptor, and are defined as members of the [Ah] gene battery. Using 14CoS/14CoS radiation deletion homozygote mice, we have localized a gene (Nmo-1n) on mouse chromosome 7 that encodes a negative effector of Nmo-1 (located on mouse chromosome 8). The Nmo-1 enzyme plays a major role in protection of animals from "oxidative stress," such as the ingestion of quinones and other phytoalexins present in foodstuff. In collaboration with A. J. Fornace, Jr., (National Cancer Institute), we have recently found three growth arrest-inducible and DNA damage-inducible (gadd) genes that are also activated in these 14CoS/14CoS radiation deletion homozygote mice, suggesting that these gadd genes are also coordinately regulated by a chromosome 7 gene encoding a negative effector. Whether Nmo-1 and the gadd genes are regulated negatively by the same gene on mouse chromosome 7 remains to be determined. This region of mouse chromosome 7--containing one or more genes encoding negative trans-acting factors--appears to resemble the recA/lexA-controlled SOS response in prokaryotes. We are attempting to clone and characterize the Nmo-1n gene and other genes encoding trans-acting factors.

Clinical applications. This Laboratory has cloned and sequenced the human CYP1A1 and CYP1A2 genes and flanking regions and have localized both genes near the MPI locus on chromosome 15. In the human population, we have shown >15-fold differences in lymphocyte CYP1A1 inducibility and in hepatic constitutive CYP1A2 gene expression. Evidence suggests that the human CYP1A1 and CYP1A2 genes, similar to the orthologous genes in laboratory animals, are important in the metabolic formation of chemical carcinogens, mutagens and teratogens. Mendelian inheritance of restriction fragment length polymorphisms (RFLPs) of the CYP1A1 gene have been determined by this Laboratory, and families with high and low cancer incidence are being studied. This Laboratory has also cloned and characterized the NMO1 gene, which presumably will also be shown to have high and low activities in the human population. In the future it should be possible to correlate RFLP patterns of these genes with human disease. Such tests would facilitate the evaluation of cancer and toxicity risk for individuals exposed to foreign chemicals. These assays would aid the individual, employer and physician in decisions regarding life style, cigarette smoking, employment, and prescription drugs.

B. A separate **Group on Microbiology**, under the direction of **Alvaro Puga, Ph.D.**, studies the various molecular mechanisms involved in CYP1A1 gene regulation. This group uses a variety of approaches to clone the Ah receptor gene, one of several transcription factors involved in CYP1A1 expression, responsible for induction of the gene by certain foreign chemicals. Two other regulatory pathways have been uncovered during the study of murine Cyp1a1 expression by this group. The first involves the enzyme poly(ADP)ribose polymerase. With the recognition site probe binding assay, a recombinant cDNA clone coding for this enzyme was isolated and shown to bind to the upstream regulatory elements of the Cyp1a1 gene. The involvement of this enzyme in Cyp1a1 gene transcription was demonstrated by using an inhibitor of poly(ADP-ribose) polymerase, which was found to repress mRNA synthesis. The second regulatory pathway was found in experiments with metabolism-deficient (P_1^-) mutant cell lines. These cells have very high, deregulated levels of Cyp1a1 mRNA synthesis and no functional Cyp1a1 enzyme, due to mutations in the structural gene. Introduction of a functional Cyp1a1 gene, or, surprisingly, the human CYP1A2 gene, into these cells restores the regulation of the endogenous Cyp1a1 gene to its constitutive levels of expression. It is clear that an autoregulatory feedback loop acts as a major component in Cyp1a1 transcription and that this regulatory mechanism involves the oxidation of an as yet unknown substrate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00136-21 LDP

PERIOD COVERED: October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Molecular Pharmacogenetics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D.W. Nebert, Head, LDP, NICHD

Others: See ATTACHMENT I

COOPERATING UNITS (if any)

See ATTACHMENT II

LAB/BRANCH: Laboratory of Developmental Pharmacology

SECTION: Section on Pharmacogenetics

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
5.59	4.17	1.42

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The cytochrome P450 (CYP) gene superfamily contains at least sixteen gene families and most likely many more. Nine of these families exist in all mammals. This laboratory has studied most extensively the CYP1 gene family. In mammals there are two tetrachlorodibenzo-p-dioxin (TCDD)-inducible genes, CYP1A1 and CYP1A2. We have examined the Cyp1a1 gene in the pSV0-cat plasmid stably transfected into mouse hepatoma Hepa-1 cultures and receptor-defective and Cyp1a1 metabolism-deficient mutant cell lines. Upstream Cyp1a1 regulatory sequences include: (a) the TATA box; (b) a proximal element between -245 and -150 that is absolutely essential for constitutive and inducible gene expression; (c) a distal element, i.e. the TCDD-inducible enhancer between -1100 and -880; and (d) an additional element that participates in a negative autoregulatory loop. Both the TCDD-inducible enhancer and the negative autoregulatory loop appear to require a functional aromatic hydrocarbon (Ah) receptor. Metabolism of endogenous substrate(s) by the Cyp1a1 gene product not only appears to control its own constitutive expression but also the expression of at least five other genes encoding enzymes with coordinate metabolic functions--Cyp1a2, NAD(P)H:menadione oxidoreductase (Nmo-1), aldehyde dehydrogenase (Aldh-1), UDP glucuronosyltransferase (Ugt-1), and glutathione transferase (Gt-1). All six of these TCDD-inducible genes have been cloned, are under control of the Ah receptor, and are defined as members of the [Ah] gene battery. The transcriptional preinitiation complex that up-regulates these genes is believed to include the Ah receptor (with foreign or endogenous ligand) and at least one other protein that confers chromatin binding capacity. Using 14CoS/14CoS radiation deletion homozygote mice, we have localized a gene (Nmo-1n) on mouse chromosome 7 that encodes a negative effector of Nmo-1 (located on mouse chromosome 8). We are attempting to clone and characterize the Ah receptor gene, the Nmo-1n gene, and other genes encoding trans-acting factors. One long-range goal of this laboratory is to develop assays, based on recombinant DNA technology, to assess the human Ah phenotype and other pharmacogenetic disorders. Such assays may predict who is at increased risk for certain types of environmentally caused birth defects, cancers, and toxicity.

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Saikh J. Haque	Guest Researcher	LDP, NICHD
John E. Jones	Staff Fellow	LDP, NICHD
Ah-Ng Kong	IRTA	LDP, NICHD
Doron Lancet	Adjunct Scientist	LDP, NICHD
Lisa A. Neuhold	Biologist (Tech.)	LDP, NICHD
Roland A. Owens	Guest Researcher	LDP, NICHD
Daniel D. Petersen	NRC BioTech Fellow	LDP, NICHD
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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00504-02 LDP

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cloning of the Ah Receptor Gene

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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COOPERATING UNITS *(if any)*

None

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SECTION

Unit on Microbiology

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NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
2.58	2.58	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Transcriptional regulation of the **CYP1A1** gene involves a number of different trans-acting factors and molecular mechanisms. Although the Ah receptor is one of the major components in the induction of CYP1A1 mRNA synthesis, several other genes are also involved. During the past year we have isolated one of these genes and we have also obtained evidence for a second, and possibly a third, major regulatory pathway in murine **Cyp1a1** gene expression. We have used recognition site binding to screen recombinant DNA expression libraries for Cyp1a1 trans-acting factors. We have isolated from one of these libraries a cDNA clone that encodes a protein that binds to the upstream region of the **Cyp1a1** gene; when sequenced, this cDNA was discovered to be mouse poly(ADP-ribose) polymerase. This enzyme is an ubiquitous, chromatin-bound protein with an obscure role in DNA repair and gene regulation. Its relevance to **Cyp1a1** gene expression was demonstrated by experiments showing that inhibition of its activity represses Cyp1a1 mRNA synthesis. A second regulatory pathway in **Cyp1a1** gene expression was uncovered in experiments with Cyp1a1 metabolism-deficient (P_1^-) mutant cell lines. These cells exhibit markedly elevated levels of Cyp1a1 mRNA but no functional Cyp1a1 enzymatic activity. Introduction of a functional **Cyp1a1** gene into these cells restores regulation of the endogenous gene, indicating that a regulatory feedback loop acts as a major component in **Cyp1a1** transcription. Most likely, this regulatory mechanism involves the oxidation of an as yet unknown endogenous substrate/ligand. Surprisingly, a an exogenously transfected functional **Cyp1a2** gene can produce the same regulatory effect upon **Cyp1a1** transcription.

LABORATORY OF MOLECULAR GENETICS (LMG)

Z01 HD 00066-19	Control Mechanisms in Temperate Bacteriophage Lambda Robert A. Weisberg, Ph.D.
Z01 HD 00067-21	Integration of Macromolecular Synthesis in Escherichia coli Michael Cashel, M.D., Ph.D.
Z01 HD 00068-18	Factors Influencing Genetics Transcription-Initiation and Termination Robert J. Crouch, Ph.D.
Z01 HD 00069-17	Molecular Genetics of Mammalian Retrovirus Replication Judith G. Levin, Ph.D.
Z01 HD 00071-17	Gene and Transgene Regulation in the Developing Mouse Heiner Westphal, M.D.
Z01 HD 01001-07	Gene Organization and Expression in Drosophila Igor B. Dawid, Ph.D.
Z01 HD 01002-07	Gene Expression During Embryonic Development of Xenopus Laevis Igor B. Dawid, Ph.D.
Z01 HD 01004-06	Regulation of Amino Acid Biosynthetic Genes in Saccharomyces Cerevisiae Alan G. Hinnebusch, Ph.D.
ZO1 HD 01005-02	Regulation of Cellular Proliferation and Diversity in Drosophila James A. Kennison, Ph.D.
ZO1 HD 01006-01	Protein-Nucleic Acid Interactions in Vertebrate Embryogenesis Thomas D. Sargent, Ph.D.

Laboratory of Molecular Genetics

Igor B. Dawid, Ph.D., Chief

During the past year the Laboratory of Molecular Genetics continued in its efforts to analyze mechanisms of the transmission, recombination and expression of the genome in a variety of organisms. These range from microorganisms to complex animals. Bacteria, yeast, and several viruses have been a focus of study. Molecular genetics originated by the study of these microorganisms, and they continue to be the most effective systems for the analysis of basic biological mechanisms. Two major programs in this area are concerned with the control of the metabolism of Escherichia coli and yeast by the environment. The complex networks that mediate this control are excellent models of regulatory mechanisms in all of biology.

An important emphasis in the Laboratory was placed on the study of developmental processes in different animal systems such as Drosophila, Xenopus and the mouse. Modern developmental biology has focused on these three animals for several reasons. The mouse is a key model for human developmental biology in view of the great similarity of embryogenesis in all mammalian species. In terms of the numbers that can be handled in genetic studies the mouse is the most suitable mammal for such work, and the establishment of many techniques for embryo manipulation and gene transfer has fostered a rapidly increasing rate of progress. There is one area, however, where studies in the mouse or any mammal are difficult: The earliest stages of embryogenesis, from fertilization through the gastrula, are comparatively inaccessible. Here the amphibian embryo has classically been the model of choice. As a vertebrate its basic mechanism of embryogenesis is similar to that in mouse or human, but early stages are accessible for manipulation and biochemical study. Thus, studies on the initial establishment of the embryonic axes and the germ layers are effectively carried out in the amphibian embryo. The animal system that combines almost all advantages for a molecular genetic study of development is the fruit fly Drosophila melanogaster. The most extensive genetics in any animal, combined with extensive studies on embryogenesis, cell lineage and, recently, molecular biology combine to make Drosophila development the best understood in the animal kingdom. Major insights valid in all animals have been obtained through the study of this organism.

The degree to which insights in one animal can be translated to another is one of the most exciting developments of recent years. Related genes are used for similar regulatory purposes in quite different animals, and structural motifs are found to recur in different proteins, utilizing a functional entity in different context. Two findings exemplify this "unity of life" theme better than most others: the homeobox and the zinc finger. Both are specific DNA-binding motifs that recur in numerous transcriptional regulatory proteins, many of which have key control functions in development. They were discovered initially in Drosophila and in Xenopus, respectively, and have since been found in all eukaryotic organisms. Work in the Laboratory in the past year has added substantially to our understanding of the

distribution, structure and function of members of these protein families. Especially exciting is the discovery of a homeobox gene in Xenopus which is responsive to mesoderm induction signals and whose function in the embryo appears to be restricted to the gastrula stage.

The research group headed by **Michael Cashel** has pursued the question how a bacterial cell regulates its gene expression during transitions between balanced growth and nutritional impoverishment. These researchers are particularly interested in the regulatory role played by the nucleotide, guanosine 3',5'-bipyrophosphate (ppGpp) as an intracellular signal to control these processes in E. coli. To analyze this question, they have dissected the genetic elements controlling ppGpp synthesis (the relA gene) and ppGpp degradation (the spoT gene). Each gene obtained in plasmids was localized, sequenced, precisely deleted, and replaced by an antibiotic resistance gene. These null alleles were placed in the chromosome by manipulations involving crossing the modified genes into a phage lambda, followed by preparing and curing phage lysogens to allow quantitation of recombinational replacement.

The results are both surprising and complex. Two routes of ppGpp synthesis have been established. One route was previously known to be associated with the RelA protein operating on a ribosome paused on an mRNA codon for lack of an available aminoacylated tRNA. When a relA deletion eliminated this route, a second route of synthesis persisted that responds only to energy source deprivation. Unexpectedly, the second route of ppGpp synthesis was abolished when the spoT gene was deleted, along with the relA gene, yielding the first prokaryotic cell devoid of detectible ppGpp. Thus, the spoT gene is either bifunctional and encodes, in addition to a ppGpp degrading activity, a ppGpp synthetic function, or spoT represses a second (still unidentified) synthetic gene. There are reasons to believe that a bifunctional protein is more likely.

Cells lacking ppGpp give clues to the functions of ppGpp. Such cells are found to be vulnerable to physiological stress; normally innocuous transient deprivations for either energy sources or amino acids become lethal as does thermal shock. In contrast, transitions from nutritionally poor to enriched conditions occur normally. Surprisingly, these cells also have cell division defects as well as growth requirements for as many as 15 of the 20 amino acids. Single step revertants that grow in the absence of amino acids have been localized to the RNA polymerase beta subunit (rpoB), showing that the auxotrophic phenotype can be overcome by modification of transcription, and that RNA polymerase is a likely target of ppGpp regulation.

The group headed by **Robert Crouch** is concerned with the structure and function of ribonuclease H (RNase H), an enzyme that degrades an RNA strand only when it is complexed with a DNA strand. Earlier work has demonstrated that E. coli cells without RNase H activity cannot survive mutations in certain recombination genes. These and other studies suggest that RNase H is important in repair and recombination. The remarkable specificity of the enzyme for the RNA strand in an RNA/DNA hybrid makes a detailed structural analysis

particularly interesting. Therefore a crystallographic study of RNase H was initiated. RNase H of E. coli, containing either methionine or selenomethionine, has been produced and crystallized. Crystals of the methionine containing RNase H have been analyzed by usual methods and shown to be in the space group $P2_12_12_1$ with cell constants of a=41.99A, b=86.68A and c=36.88A. Crystals of the selenomethionine containing RNase H have been obtained and data collected by MAD.

The retrovirus pol gene encodes a protein that has reverse transcriptase and RNase H activity. The retroviral RNase H has been studied in collaboration with Dr. Levin. Retroviral RNase H has long been thought to be an exonuclease. The enzymatic nature of the RNase H has been reexamined on certain well defined substrates. RNase H could degrade RNA of RNA:DNA hybrids even when the DNA was more than 200 nucleotides from either the 3' or 5' termini. This result is consistent with the fact that the same hybrids could serve as primers for DNA synthesis but were at odds with the idea that the RNase H acted in an exonucleolytic manner. Further proof that RNase H acts endonucleolytically was obtained by constructing a circular RNA of about 170 bases which was annealed to a complementary 20 base oligomeric DNA. This substrate was also cleaved by the retroviral RNase H.

A group headed by **Igor Dawid** is concerned with molecular mechanisms during early amphibian development. As in all vertebrates, the frog embryo generates its different tissue distributions in an orderly process through a series of cell-cell interactions called embryonic induction. The profound influence of induction on the developmental fate of embryonic cells had been known for many years, but only recently has there been progress in analyzing the molecular basis of these events. A role for growth factors, specifically of the TGF-β family, has been suggested in mesoderm induction. Using the mesoderm inducing factor (MIF) from XTC cells, the activation of several immediate early genes in the responding tissue has been demonstrated. One of these genes encodes a homeodomain protein and was named Mix.1; it is expressed in the vegetal region during a short period in blastula and gastrula embryos and may be involved in the establishment of mesoderm and endoderm.

In a related project the development of the nervous system in Xenopus was studied by the isolation of neurospecific genes that are activated during gastrula stages. Five genes in this class were identified by sequencing. They include a β-tubulin, a homolog of the β-subunit of Na/K ATPase, and a gene encoding a putative RNA-binding protein. Especially the latter finding is of considerable interest since RNP proteins may be involved in RNA processing and transport, raising the possibility of studying tissue-specific control of RNA metabolism in the brain.

The other major DNA-binding motif in addition to the homeodomain is the zinc finger. In addition to structural studies on the original finger protein, Xenopus TFIIIA, an analysis of finger-containing genes in the human genome was undertaken. Preliminary evidence was obtained that the genome contains many such genes, at least 40 and possibly well

beyond 100.

Using the same model system, Xenopus laevis, the group headed by **Tom Sargent** aims to identify informational molecules that are localized in the egg, thereby assigning different fates to different regions in the early embryo. Such localized informational molecules have been postulated since the last century, but direct evidence for their nature has been lacking. To study this issue Dr. Sargent and his colleagues attempt to characterize proteins that regulate gene expression in vertebrate embryos in a regionally specific manner. The primary model system is based on the transcriptional regulation of an epidermal keratin gene, XK81A1, in Xenopus embryos. The cis-regulatory elements of this gene have been mapped using transgenic frog embryos as an assay system. One regulatory site has been precisely located and characterized in detail. A positive transcription factor, KTF-1, binds to this DNA and stimulates the activity of the keratin promoter. Characterization and cloning of KTF-1 are currently being pursued, and other positive and negative regulatory elements in the keratin promoter are being investigated.

In addition, other developmentally interesting genes have been recovered from two subtracted cDNA libraries prepared as part of this program: UVS.1 and UVS.2 (for ultraviolet sensitive) are expressed specifically in cement gland and hatching gland, respectively. These and other genes in this series are markers for dorsoanterior axis formation. The gene DG72 is expressed specifically in the posterior region of the embryo from gastrulation through early organogenesis. This gene is inducible with the mesoderm inducer XTC-MIF, and the predicted protein sequence includes a region similar to the "zinc finger-like" RNA binding domain of retroviral gag proteins as well as an extended acidic helix with a short "leucine zipper". The regulation and function of these genes are subjects for ongoing and future investigation.

Three groups are interested in the molecular genetics of Drosophila. Work directed by **Igor Dawid** is concerned with the homeotic regulatory genes fsh and trithorax (trx). The fsh gene is a maternal effect locus which leads to homeotic transformations in the progeny of mutant females; the frequency of transformations is greatly increased by interaction between mutations in fsh and trx. The major products of fsh have been identified as two large proteins with membrane-spanning domains. Antisera produced against fusion proteins show that the primary translation products are processed to three smaller proteins, and preliminary evidence for glycosylation of the fsh proteins has been obtained. Analysis of progeny of fsh mutant females shows distorted expression in gap, segmentation and homeotic genes as a probable basis for the developmental abnormalities in these embryos. In particular, the domain of expression of the gap gene Krüppl is diminished, showing a very early effect of maternal fsh deficiency on embryonic gene expression.

The trithorax (trx) gene, a major regulatory locus in Drosophila, has been cloned previously. Sequence analysis of cDNAs predicts a very large protein of molecular mass 400,000 as the major product of trx.

This protein contains cysteine-rich regions that are hypothesized to be zinc-dependent nucleic acid-binding domains.

Studies carried out by **Susan Haynes** have focused on the genes encoding RNA-binding proteins in Drosophila. Such proteins are of great interest since they may be involved in RNA processing and transport, offering opportunities for regulation of gene expression in the cell. Evidence from various sources has accumulated in recent times that such control is important in development and cell physiology. In Haynes' work a family of genes encoding hnRNP proteins, the Hrb family, has been identified in Drosophila. Two members of this gene family have been analyzed in detail. The Hrb98DE locus produces eight different RNAs by alternative splicing. This gene is expressed throughout development, but the contributions of the different RNAs vary. The Hrb32AB locus gives rise to two RNAs by the use of different polyadenylation sites. Antisera produced against fusion proteins detect multiple bands in fly extracts. The antigens comigrate with nuclear RNA in sedimentation analysis, as predicted for hnRNP proteins. Ongoing work is aimed at generating mutations in the Hrb loci for an analysis of the in vivo functions of these genes and their products.

The group headed by **Jim Kennison** is interested in the genetic control of establishment of the body plan in Drosophila. Genes involved in the determination of segmental identity have been identified on the basis of genetic interactions with homeotic mutations, mutations already known to affect the process. Of the eighteen genes identified by interacting mutations, three act as negative regulators of homeotic genes, and the remaining fifteen appear to be positive regulators, ancillary factors, or targets of homeotic gene function. Of the latter fifteen, the genes kismet and Sex combs reduced behave as targets for the homeotic gene Antennapedia. The genes brahma, osa, moira, skuld, kohtalo, and sallimus behave as ancillary factors for Antennapedia function, with at least brahma also acting as a positive regulator of Antennapedia gene function. Mutations associated with insertions of a Drosophila transposable element have been isolated for the genes kismet, brahma, and osa. The sequence of the putative protein product of the brahma locus has been determined.

A 55 kilobase region of the genome including the brahma locus has been characterized by molecular and genetic methods. Three transcription units in addition to the brahma locus have been identified within this region of DNA. Sequence analyses of cDNAs from two of the three transcription units predict putative protein products with extensive sequence similarities to mammalian proteins thought to be involved in signal transduction. One putative protein product has similarities to the catalytic subunit of cAMP-dependent protein kinase, while the other putative protein product is similar to a GTP-binding protein termed ARF (ADP-ribosylation factor). Approximately thirty-five mutations in essential genes in this chromosome region have been isolated. These mutations identify at least three genes in addition to the brahma locus.

Alan Hinnebusch and his colleagues are involved in a program to study

the elaborate regulatory network that allows yeast cells to respond to the availability of amino acids in the medium. When amino acids are lacking the cells turn up the production of the various enzyme systems that synthesize amino acids. However, when these nutrients are plentiful in the medium it is of considerable selective advantage for the cell to turn off the production of such enzymes which would be a waste of resources under these conditions. A hierarchy of regulatory genes mediates this control in yeast; various mechanisms are involved, including transcriptional and translational regulation and protein modification by kinase action. The proximal factor in the hierarchy is GCN4, a transcriptional activator of amino acid biosynthetic genes that is itself regulated at the translational level by short open reading frames (uORFs) present in the leader of GCN4 mRNA. The mechanism of translational regulation of GCN4 expression has been a major focus of research of this group, both because of its intrinsic interest and as an example of translational regulation in eukaryotes in general. uORFs 3 and 4 repress GCN4 expression in nonstarvation conditions, whereas uORF1 stimulates GCN4 expression by overcoming the inhibitory effects of uORFs 3 and 4. The distinct functions of uORFs 1 and 4 are determined by sequences immediately surrounding their stop codons, suggesting that translation termination occurs differently at these two sites. Reinitiation after uORF4 translation is very inefficient in nonstarvation conditions; by contrast, uORF1 translation is compatible with downstream initiation and this property is important for its ability to regulate uORF4. Translation of uORF1 may alter the ribosome in a way that facilitates its advance through uORF4 sequences in amino acid-starved cells. Positive and negative trans-acting factors are also required to regulate ribosome movement in the GCN4 mRNA leader. Modulating the activity of the general translation initiation factor eIF-2 appears to be one important function of the positive regulators GCN2 and GCN3. At least two negative regulators of GCN4 expression, GCD1 and GCD2, are essential factors that probably also function in translation initiation. Interestingly, GCD1 and GCD2 appear to exist in a complex with GCN3, suggesting that GCN3 directly modulates the activity of these gene products. GCN2 stimulates GCN4 expression by functioning as a protein kinase. Homology between the C-terminus of GCN2 and histidyl-tRNA synthetases suggests that GCN2 detects amino acid starvation by directly monitoring the concentration of uncharged tRNA. In this model, tRNA binding stimulates GCN2 kinase activity. Mutations in GCD genes, in genes encoding subunits of eIF-2, and in GCN3 have been identified that all bypass the requirement for GCN2 to derepress GCN4 expression, making these factors potential substrates for phosphorylation by GCN2 in amino acid-starved cells.

Ribosomal protein (rp) genes are repressed under the same amino acid starvation conditions that lead to elevated expression of amino acid biosynthetic genes under GCN4 control. Repression of the RPL16A rp gene requires regulatory sequences that include the binding site for the transcriptional activator TUF. Regulation of RPL16A expression occurs independently of GCN factors, indicating that separate pathways exist for these two transcriptional responses to amino acid starvation.

The goal of the program headed by **Judith Levin** is to define the molecular mechanisms involved in the replication of mammalian

retroviruses and in particular, to understand the factors which influence the regulation and expression of viral genetic information. Studies are being carried out with the murine leukemia virus system. Current interest is focused on the organization of the reverse transcriptase coding region and on the identification of functionally significant sequences within the RNase H domain. Molecular clones containing reverse transcriptase sequences are being expressed in E. coli. These clones include one which contains the entire coding region as well as constructs in which the gene has been modified by deleting (i) the RNase H domain; (ii) the polymerase domain; and (iii) sequences within or immediately upstream of the RNase H domain. The results confirm the previous assignment of the RNase H domain to the carboxyl-terminal region of reverse transcriptase. Thus, RNase H activity is lost by deleting all or a substantial portion of this region, but is retained when the deletion is in an upstream sequence. Studies are in progress to determine the polymerase activity of these constructs and to evaluate the functional relationship between the polymerase and RNase H domains.

A second project of this group deals with translational control of viral gene expression. In retroviruses, termination codons are found at the gag-pol junction which do not, however, lead to a complete stop of translation at this position. Different mechanisms like read-through suppression and frame shifting, occur in different viruses to allow the modulated continuation of translation through this region. Dr. Levin and her colleagues have focused on the role of nucleotide context in suppression of the in-frame UAG codon at the MuLV gag-pol junction. A short 66-base sequence consisting almost entirely of nucleotides downstream of the termination codon has been shown to provide a sufficient context for readthrough suppression. In addition, in vitro and in vivo experiments showing suppression with constructs containing UAA or UGA instead of UAG indicate that (i) the signal inducing suppression is not unique for UAG; (ii) mammalian cells and cell extracts contain tRNAs capable of translating UAA and UGA as amino acids; and (iii) UAA is not an absolute termination signal in higher eukaryotes (as originally thought). Experiments to identify the precise signals utilized by the virus to mediate suppression, including the potential role of mRNA secondary structure and the involvement of ribosomal pausing in misreading of the termination codon by suppressor tRNA, are underway.

Robert Weisberg and his colleagues have continued their interest in the study of gene expression and recombination, using the lambdoid bacteriophages as their system. As a result of extensive studies by many laboratories our understanding of the molecular mechanisms controlling the life cycle of this group of viruses may be the best understood of any biological entity. Recent work in Dr. Weisberg's group has focused on the comparative analysis of gene expression and recombination in the related phages HK022 and λ. Gene expression in the temperate coliphage HK022 is similar to that in other lambdoid phages: the genes that are expressed early after infection are organized into two divergently transcribed operons, and the initiation of transcription from the corresponding promoters (pL and pR) is blocked by the phage repressor. However, HK022 early transcription also has

novel features. First, the two early operons are transcribed poorly when phage DNA synthesis is blocked. Since pL and pR are strong promoters in vitro, it appears that some factor or condition missing in vitro limits transcription in vivo. Second, HK022, in contrast to its relatives, does not encode a factor that promotes antitermination of early transcription. Nevertheless, antitermination of early transcription occurs in HK022, and requires specific sites located in the early operons. Thus, antitermination in this phage is either factor-independent or the required factors are encoded by the host. Third, HK022 expresses nun, the first gene of the pL operon, in the presence of phage repressor. Nun transcription in these conditions originates not at pL but at an upstream promoter. The nun protein protects HK022 lysogens from superinfection with phage λ by terminating transcription at the λ nut sites. The specific nut sequences required for nun termination overlap but do not completely coincide with those required for antitermination promoted by the λ N protein.

HK022 and λ both encode proteins that promote recombination between special DNA sequences called attachment sites. The mechanism of site-specific recombination in the two phages is very similar, but the sites and one of the proteins (the Int protein) are not interchangeable. In order to localize the determinants that distinguish these two recombination systems, hybrid int genes were constructed and attachment sites and their function analyzed. The determinants of protein specificity are located amino terminal of Int residue 281. This segment does not contain the highly conserved "recombination domain" of the Integrase family proteins. The specificity determinants of the attachment sites are located in the regions previously identified as "core type" Int binding sites in λ by Ross and Landy. The segments corresponding to the C and B' λ core Int binding sites appear to be especially important, and the C' and B sites to be less important. The arms of the phage attachment site, which contain IHF, Xis and "arm-type" Int binding sites, do not determine the specificity difference.

Fundamental questions of development, differentiation and oncogenesis in man are best answered by analyzing parallel processes in laboratory mice. **Heiner Westphal** and his colleagues have utilized this system in different ways to gain insights into the understanding of mechanisms of gene control in the intact mammalian organism.

The first of a number of projects addressed questions of early embryonic development. Specific spatial and temporal patterns of expression were established for individual members of the homeobox and myc gene families. Factors encoded by these genes have been implicated in the transcriptional regulation of early pattern formation in the developing embryo. In situ analysis performed with tissue sections of the postimplantation embryo have revealed distinct spatial and temporal patterns of expression of several members of these genes. Both the homeobox and the myc gene families are likely to be involved in orchestrating individual facets of early mouse development. Presently, Westphal's laboratory is involved in establishing very demanding techniques aimed at generating specific gene defects in mice. Defective DNA sequences are being introduced via homologous recombination in specific target genes of pluripotent mouse embryonic stem cells. These

cells will be injected in blastocysts in an effort to generate mutations in specific chromosomal genes of the mouse germ line. If successful, this type of experiment will corroborate genetic evidence for the involvement of homeobox, myc, and other gene families in the regulation of mouse development. It will also be used by Westphal's laboratory to generate mouse models of human genetic diseases.

In another project, Westphal and his coworkers utilized the simple architecture of the eye lens to develop a model system for the analysis of oncogenesis in the living mammalian organism. Oncogene products of polyoma and SV40 virus released in lens tissue at specific times of mouse development resulted in distinct lens pathologies. These findings lead to the intriguing postulate that both timing of expression and the dose of a given oncogene product are of crucial importance for subsequent steps of growth deregulation.

Finally, Westphal's laboratory has continued to its work in the field of biomedical research. The placenta was identified as an organ that is very active in recognizing AIDS virus promoters in the transgenic mouse. This result has important implications for the risk assessment for children born to mothers infected with the human immunodeficiency virus. Also, the laboratory has begun to exploit possibilities of producing in milk of transgenic farm animals large amounts of tissue plasminogen activator and of sCD4, human proteins needed to treat coronary diseases and AIDS, respectively.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00066-19 LMG

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October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Control Mechanisms in Temperate Bacteriophage Lambda

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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COOPERATING UNITS *(if any)* Institute of Cancer Research; Columbia University, NY (Max Gottesman); Department of Biochemistry, University of Arizona, Tucson (John Little); Department of Biochemistry, Tel Aviv University, Israel (Ezra Yagil)

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Microbial Genetics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Gene expression in the temperate coliphage HK022 is similar to that in other lambdoid phages: the genes that are expressed early after infection are organized into two divergently transcribed operons, and the initiation of transcription from the corresponding promoters (pL and pR) is blocked by the phage repressor. However, HK022 early transcription also has novel features. First, the two early operons are transcribed poorly when phage DNA synthesis is blocked. Since pL and pR are strong promoters *in vitro*, it appears that some factor or condition missing *in vitro* limits transcription *in vivo*. Second, HK022, in contrast to its relatives, does not encode a factor that promotes antitermination of early transcription. Nevertheless, antitermination of early transcription occurs in HK022, and requires specific sites located in the early operons. Thus, antitermination in this phage is either factor-independent or the required factors are encoded by the host. Third, HK022 expresses *nun*, the first gene of the pL operon, in the presence of phage repressor. *Nun* transcription in these conditions originates not at pL but at an upstream promoter. The Nun protein protects HK022 lysogens from superinfection with phage lambda by terminating transcription at the lambda *nut* sites. The specific *nut* sequences required for Nun termination overlap but do not completely coincide with those required for antitermination promoted by the lambda N protein.

HK022 and lambda both encode proteins that promote recombination between special DNA sequences called attachment sites. The mechanism of site-specific recombination in the two phages is very similar, but the sites and one of the proteins (the Int protein) are not interchangeable. In order to localize the determinants that distinguish these two recombination systems, we constructed hybrid *int* genes and attachment sites and analyzed their function. The determinants of protein specificity are located amino terminal of Int residue 281. This segment does not contain the highly conserved "recombination domain" of the Integrase family proteins. The specificity determinants of the attachment sites are located in the regions previously identified as "core type" Int binding sites in lambda by Ross and Landy. The segments corresponding to the C and B' lambda core Int binding sites appear to be especially important, and the C' and B sites to be less important. The arms of the phage attachment site, which contain IHF, Xis and "arm-type" Int binding sites, do not determine the specificity difference.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00067-21 LMG

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Integration of Macromolecular Synthesis in Escherichia coli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C.M. Cashel	Section Head	LMG, NICHD
Others:	H. Xiao	Visiting Fellow	LMG, NICHD
	M. Kalman	Visiting Scientist	LMG, NICHD
	I. Szeverenyi	Visiting Fellow	LMG, NICHD

COOPERATING UNITS *(if any)*
Dept. Cellular Biochemistry, Hadassah Medical School, Jerusalem, Israel
(Gad Glaser)

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Molecular Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.2	3.2	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The overall purpose of this project is to understand cellular mechanisms which coordinate the gene expression during transitions between balanced growth and nutritional impoverishment. We are particularly interested in the regulatory role played by the nucleotide, guanosine 3',5'-bispyrophosphate (ppGpp) as an intracellular signal to control these processes in E. coli.

We have dissected the genetic elements controlling ppGpp synthesis (the relA gene) and ppGpp degradation (the spoT gene). Each gene in plasmids was localized, sequenced, precisely deleted, and replaced by an antibiotic resistance gene. These null alleles were placed in the chromosome by manipulations involving crossing the modified genes into a phage lambda, preparing and curing phage lysogens to allow quantitation of recombinational replacement.

The results are both surprising and complex. Two routes of ppGpp synthesis have been proven. One route was previously known to be associated with the RelA protein operating on a ribosome paused on a mRNA codon for lack of an available aminoacylated tRNA. When a relA deletion eliminated this route, a second route of synthesis persisted that responds only to energy source deprivation. Unexpectedly, the second route of ppGpp synthesis was abolished when the spoT gene was deleted, along with the relA gene, yielding the first prokaryotic cell devoid of detectible ppGpp. Thus, the spoT gene is either bifunctional and also encodes a ppGpp synthetic function or represses a second (still unidentified) synthetic gene. We have reasons to believe that a bifunctional protein is more likely.

Cells lacking ppGpp give clues to the functions of ppGpp. Such cells are found to be vulnerable to physiological stress; normally innocuous transient deprivations for either energy sources or amino acids become lethal as does thermal shock. In contrast, transitions from nutritionally poor to enriched conditions occur normally. Surprisingly, these cells also have cell division defects as well growth requirements for as many as 15 of the 20 amino acids. Single step revertants that grow in the absence of amino acids have been localized to the RNA polymerase beta subunit (rpoB), showing that the the auxotrophic phenotype can be overcome by modification of transcription and that RNA polymerase is a likely target of ppGpp regulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00068-18 LMG

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Factors Influencing Genetics Transcription-Initiation and Termination

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.J. Crouch	Research Chemist	LMG, NICHD
Others:	L. Lempereur	Visiting Fellow	LMG, NICHD
	D.-Y. Shin	Visiting Fellow	LMG, NICHD
	M.-L. Dirksen	Biologist	LMG, NICHD
	E. Kalman	Visiting Associate	LMG, NICHD
	D. Seay	Student Trainee (Biology)	LMG, NICHD
	M. Kavuru	Special Volunteer	LMG, NICHD
	J. Levin	Research Biochemist	LMG, NICHD

COOPERATING UNITS *(if any)*
Mitsubishi Institute of Life Sciences, Tokyo (T. Uchida); Columbia University, New York (W. Hendrickson).

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Unit on Formation of RNA

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
3.7	2.6	1.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major area of research reported here is the study of the structure and function of ribonuclease H (RNase H). RNase H of E. coli, containing either methionine or selenomethionine has been produced and crystallized. Crystals of the methionine containing RNase H have been analyzed by usual methods and shown to be in the space group P212121 with cell constants of a=41.99A, b=86.68A and c=36.88A. Crystals of the selenomethionine containing RNase H have been obtained and data collected by MAD. Retroviral reverse transcriptase RNase H has long been thought to be an exonuclease. We have reexamined the enzymatic nature of the RNase H on some well defined substrates. We found that the RNase H could degrade RNA of RNA:DNA hybrids even when the DNA was more than 200 nucleotides from either the 3' or 5' termini. This result is consistent with the fact that the same hybrids could serve as primers for DNA synthesis but were at odds with the idea that the RNase H acted in an exonucleolytic manner. Further proof that the RNase H acts endonucleolytically was obtained when we constructed a circular RNA of about 170 bases which was annealed to a complementary 20 base oligomeric DNA. This substrate was also cleaved by the retroviral RNase H. Retroviral reverse transcriptase (RT) is a single polypeptide having both polymerase and RNase H activities. We have further defined the limits of the RNase H domain by making deletions within the region between the polymerase and RNase H domains which do not inactivate the RNase H activity. Deletion of the entire RNase H domain, of course, destroys the RNase H activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00069-17 LMG

PERIOD COVERED: October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*: Molecular Genetics of Mammalian Retrovirus Replication

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	J.G. Levin	Research Biochemist	LMG, NICHD
Others:	Y.-X. Feng	Visiting Associate	LMG, NICHD
	K. Post	Biologist	LMG, NICHD
	H. Nguyen	SIS	LMG, NICHD
	R. Crouch	Research Chemist	LMG, NICHD

COOPERATING UNITS *(if any)*: NCI-LEC (D. Hatfield); BRI Basic Research Program, NCI-FCRF (A. Rein); PRI-FCRF (M. Zweig)

LAB/BRANCH: Laboratory of Molecular Genetics

SECTION: Unit on Viral Gene Regulation (Developmental Biology Section)

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.1	2.0	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The goal of this project is to define the molecular mechanisms involved in the replication of mammalian retroviruses and in particular, to understand the factors which influence the regulation and expression of viral genetic information. Studies are being carried out with the murine leukemia virus system. Current interest is focused on the organization of the reverse transcriptase coding region and on the identification of functionally significant sequences within the RNase H domain. Molecular clones containing reverse transcriptase sequences are being expressed in E. coli. These clones include one which contains the entire coding region as well as constructs in which the gene has been modified by deleting (i) the RNase H domain; (ii) the polymerase domain; and (iii) sequences within or immediately upstream of the RNase H domain. The results confirm our previous assignment of the RNase H domain to the carboxyl-terminal region of reverse transcriptase. Thus, RNase H activity is lost by deleting all or a substantial portion of this region, but is retained when the deletion is in an upstream sequence. Studies are in progress to determine the polymerase activity of these constructs and to evaluate the functional relationship between the polymerase and RNase H domains. In other work, translational control of viral gene expression is being investigated. Efforts are focused on the role of nucleotide context in suppression of the in-frame UAG codon at the MuLV gag-pol junction. A short 66-base sequence consisting almost entirely of nucleotides downstream of the termination codon has been shown to provide a sufficient context for readthrough suppression. In addition, in vitro and in vivo experiments showing suppression with constructs containing UAA or UGA instead of UAG indicate that (i) the signal inducing suppression is not unique for UAG; (ii) mammalian cells and cell extracts contain tRNAs capable of translating UAA and UGA as amino acids; and (iii) UAA is not an absolute termination signal in higher eukaryotes (as originally thought). Experiments to identify the precise signals utilized by the virus to mediate suppression, including the potential role of mRNA secondary structure and the involvement of ribosomal pausing in misreading of the termination codon by suppressor tRNA, are underway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00071-17 LMG

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Gene and Transgene Regulation in the Developing Mouse

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principel Investigator.) (Name, title, laboratory, and institute affilietion)*

PI: H. Westphal, Head, LMG, NICHD

(See attachment)

COOPERATING UNITS *(if eny)*

(See attachment)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Mammalian Gene Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
13.1	9.6	3.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Molecular genetics of mammalian development, differentiation and oncogenesis. This part of the project contains two approaches.

A. Homeobox genes and myc genes. Factors encoded by these families of genes have been implicated in the transcriptional regulation of embryonic development. In situ hybridization of embryonic tissue sections and whole mounts of embryos with polymerase chain reaction analysis have revealed distinct spatial and temporal patterns of expression of several members of these genes. Both the homeobox and the myc gene families are likely to be involved in orchestrating individual facets of early mouse development. Presently, we attempt to introduce defective DNA sequences via homologous recombination into specific target genes of pluripotent mouse embryonic stem cells in an effort to generate mutations in specific chromosomal genes. If successful, this type of experiment will corroborate genetic evidence for the involvement of homeobox, myc, and other gene families in the regulation of mouse development. It can also be used to generate mouse models of human genetic diseases.

B. Differentiation and oncogenesis in the eye lens of transgenic mice. We have utilized the simple architecture of the eye lens to develop a model system for the analysis of deregulated growth in the living mammalian organism. Oncogene products of polyoma and SV40 virus released in lens tissue at specific times of mouse development result in distinct lens pathologies. Our findings have allowed us to propose a working model postulating that both timing of expression and the dose of a given oncogene product are of crucial importance for subsequent steps of growth deregulation.

Biomedical research. In this part of our project, we have identified the placenta as an organ that is very active in recognizing AIDS virus promoters in the transgenic mouse. This result has important implications for the risk assessment for children born to mothers infected with the human immunodeficiency virus. Also, we have begun to exploit possibilities of producing in milk of transgenic farm animals large amounts of tissue plasminogen activator and of sCD4, human proteins needed to treat coronary diseases and AIDS, respectively.

Professional personnel:

L. Crofford, Medical Staff Fellow
E.M. Fuchtbauer, Visiting Fellow
A. Griep, Senior Staff Fellow
S.-P. Huang, Chemist
M. Johnson, Medical Staff Fellow
E. Lee, Veterinarian
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K. Mahon, Senior Staff Fellow
A. Miller, Biologist
B. Mosinger, Visiting Fellow
T. Nakamura, Visiting Fellow
U. Tillmann, Guest Researcher
M. Tremblay, Guest Researcher
Y. Yaginuma, Visiting Fellow
S. Yu, Visiting Associate
L. Yuan, Biologist

Cooperating units:

NEI (T. Kuwabara)
NEI (J. Piatigorsky)
NICHD (K. Ozato)
Einstein U., NY (R. DePinho)
The Weizmann Inst. of Science, Rehovot, Israel (R. Miskin)
E.I. duPont de Nemours & Co., Wilmington, DE (B. Sauer)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01001-07 LMG

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gene Organization and Expression in Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	I. Dawid	Head	LMG, NICHD
Others:	S. Haynes	Senior Staff Fellow	LMG, NICHD
	N. Bhatia-Dey	Visiting Fellow	LMG, NICHD
	D.-H. Huang	Visiting Associate	LMG, NICHD
	A. Mazo	Visiting Scientist	LMG, NICHD
	J. Kennison	Senior Staff Fellow	LMG, NICHD

COOPERATING UNITS *(if any)*
Department of Microbiology, University of Virginia (Ann Beyer)

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Developmental Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.25	4.0	0.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major products of the maternal effect homeotic gene fsh of Drosophila have been identified as two large proteins with membrane-spanning domains. Antisera produced against fusion proteins show that the primary translation products are processed to three smaller proteins. Supportive evidence for glycosylation of the fsh proteins was also obtained. Analysis of progeny of fsh mutant females shows distorted expression in gap, segmentation and homeotic genes as a probable basis for the developmental abnormalities in these embryos. In particular, the domain of expression of the gap gene Krüppl is diminished, showing a very early effect of maternal fsh deficiency on embryonic gene expression.

The trithorax (trx) gene, a major regulatory locus in Drosophila, has been cloned previously. Sequence analysis of cDNAs predicts a very large protein of molecular mass 400,000 as the major product of trx. This protein contains cysteine-rich regions that are hypothesized to be zinc-dependent nucleic acid-binding domains.

A family of genes encoding hnRNP proteins, the Hrb family, has been identified in Drosophila. Two members of this gene family have been analyzed in detail. The Hrb98DE locus produces eight different RNAs by alternative splicing. This gene is expressed throughout development, but the contributions of the different RNAs vary. The Hrb32AB locus gives rise to two RNAs by the use of different polyadenylation sites. Antisera produced against fusion proteins detect multiple bands in fly extracts. The antigens comigrate with nuclear RNA in sedimentation analysis, as predicted for hnRNP proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01002-07 LMG

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gene Expression During Embryonic Development of Xenopus Laevis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: I.B. Dawid, Head (All personnel listed below associated with LMG/NICHD)

Others:
G. Michaels, Staff Fellow
F. Rosa, Visiting Fellow
N. Bhatia-Dey, Visiting Fellow
P. Bray, IRTA
P. Good, IRTA
R. Friesel, NRC Fellow
M. Rebbert, Chemist

COOPERATING UNITS *(if any)*
Basel Inst. Immunology, Switzerland (H.-J. Thiesen); LC, DCE, NCI (A. Roberts & M. Sporn); DCRT (B. Brooks and R. Feldman)

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Developmental Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.75	6.75	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This work aims to elucidate molecular events during early amphibian embryogenesis, with a particular emphasis on embryonic induction. The role of growth factors in induction was investigated through analysis of transforming growth factor beta (TGF-beta) secreted by the XTC cell line. Two forms, TGF-beta2 and TGF-beta5, were isolated from XTC culture fluid and identified by sequencing. Xenopus TGF-beta2 cDNA has been cloned and sequenced. The immediate early response to mesoderm induction was analyzed by isolating rapidly activated genes. One of these genes encodes a homeodomain protein and was named Mix.1; it is expressed in the vegetal region during a short period in blastula and gastrula embryos and may be involved in the establishment of mesoderm and endoderm. The development of the nervous system in Xenopus was studied by the isolation of neurospecific genes that are activated during gastrula stages. Five genes in this class were identified by sequencing. They include a beta-tubulin, a homolog of the beta-subunit of Na/K ATPase, and a gene encoding a putative RNA-binding protein.

The structure of zinc finger domains was studied by computer simulation and physical methods. Also, finger homologs were isolated from a human DNA library to gauge the extent of distribution of such genes in the genome. Some 200 clones with finger homologies have been obtained, suggesting that a large family of related sequences is present in the genome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01004-06 LMG

PERIOD COVERED: October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Regulation of Amino Acid Biosynthetic Genes in Saccharomyces Cerevisiae

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: A.G. Hinnebusch, Research Microbiologist, (All listed personnel LMG/NICHD)

Others:	P. Miller	NRC Fellow	D. Crouch	Guest Researcher
	N. Williams	Guest Researcher	R. Wek	IRTA Fellow
	E. Hannig	IRTA Fellow	J.P. Abastado	Visiting Fellow
	C. Paddon	Visiting Associate	C. Moehle	NRC Fellow
	B. Jackson	Biologist	M. Cigan	NRC Fellow
	M. Foiani	Visiting Fellow	M. Ramirez	Visiting Fellow

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Unit on Molecular Genetics of Lower Eukaryotes (Section on Developmental Biology)

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
9.92	8.92	1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

GCN4 is a transcriptional activator of amino acid biosynthetic genes that is itself regulated at the translational level by short open reading frames (uORFs) present in the leader of GCN4 mRNA. uORFs 3 and 4 repress GCN4 expression in nonstarvation conditions, whereas uORF1 stimulates GCN4 expression by overcoming the inhibitory effects of uORFs 3 and 4. The distinct functions of uORFs 1 and 4 are determined by sequences immediately surrounding their stop codons, suggesting that translation termination occurs differently at these two sites. Reinitiation after uORF4 translation is very inefficient in nonstarvation conditions; by contrast, uORF1 translation is compatible with downstream initiation and this property is important for its ability to regulate uORF4. We propose that translation of uORF1 alters the ribosome in a way that facilitates its advance through uORF4 sequences in amino acid-starved cells. Positive and negative trans-acting factors are also required to regulate ribosome movement in the GCN4 mRNA leader. Modulating the activity of the general translation initiation factor eIF-2 appears to be one important function of the positive regulators GCN2 and GCN3. At least two negative regulators of GCN4 expression, GCD1 and GCD2, are essential factors that probably also function in translation initiation. Interestingly, GCD1 and GCD2 appear to exist in a complex with GCN3, suggesting that GCN3 directly modulates the activity of these gene products. GCN2 stimulates GCN4 expression by functioning as a protein kinase. Homology between the C-terminus of GCN2 and histidyl-tRNA synthetases suggests that GCN2 detects amino acid starvation by directly monitoring the concentration of uncharged tRNA. In this model, tRNA binding stimulates GCN2 kinase activity. Mutations in GCD genes, in genes encoding subunits of eIF-2, and in GCN3 have been identified that all bypass the requirement for GCN2 to derepress GCN4 expression, making these factors potential substrates for phosphorylation by GCN2 in amino acid-starved cells.

Ribosomal protein (rp) genes are repressed under the same amino acid starvation conditions that lead to elevated expression of amino acid biosynthetic genes under GCN4 control. Repression of the RPL16A rp gene requires regulatory sequences that include the binding site for the transcriptional activator TUF. Regulation of RPL16A expression occurs independently of GCN factors, indicating that separate pathways exist for these two transcriptional responses to amino acid starvation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01005-02 LMG

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Cellular Proliferation and Diversity in Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Kennison	Senior Staff Fellow	LMG, NICHD
Others:	A. Felsenfeld	NRC Biotech Fellow	LMG, NICHD
	B. Judge	Biologist (Tech)	LMG, NICHD
	J. Ballard	SIS	LMG, NICHD

COOPERATING UNITS *(if any)*
Department of Biology, University of California, Santa Cruz, California (John Tamkun); LMC, NCI, NIH (Richard Kahn)

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Developmental Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.0	1.6	1.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genes involved in the determination of segmental identity in Drosophila melanogaster have been identified on the basis of genetic interactions with homoeotic mutations, mutations already known to affect the process. Of the eighteen genes identified by interacting mutations, three act as negative regulators of homoeotic genes, and the remaining fifteen appear to be positive regulators, ancillary factors, or targets of homoeotic gene function. Of the latter fifteen, the genes kismet and Sex combs reduced behave as targets for the homoeotic gene Antennapedia. The genes brahma, osa, moira, skuld, kohtalo, and sallimus behave as ancillary factors for Antennapedia function, with at least brahma also acting as a positive regulator of Antennapedia gene function. Mutations associated with insertions of a Drosophila transposable element have been isolated for the genes kismet, brahma, and osa. The sequence of the putative protein product of the brahma locus has been determined.

A 55 kilobase region of the genome including the brahma locus has been characterized by molecular and genetic methods. Three transcription units in addition to the brahma locus have been identified within this region of DNA. Sequence analyses of cDNAs from two of the three transcription units predict putative protein products with extensive sequence similarities to mammalian proteins thought to be involved in signal transduction. One putative protein product has similarities to the catalytic subunit of cAMP-dependent protein kinase, while the other putative protein product is similar to a GTP-binding protein termed ARF (ADP-ribosylation factor). Approximately thirty-five mutations in essential genes in this chromosome region have been isolated. These mutations identify at least three genes in addition to the brahma locus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01006-01 LMG

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Protein-Nucleic Acid Interactions in Vertebrate Embryogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. Sargent	Senior Staff Fellow	LMG/NICHD
Others:	E. Jonas	Visiting Associate	LMG/NICHD
	A. Snape	Visiting Fellow	LMG/NICHD
	S. Sato	Senior Staff Fellow	LMG/NICHD

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Developmental Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.5	3.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The aim of this project is to characterize proteins that bind and regulate the expression of DNA and RNA molecules in vertebrate embryos as an approach to understanding the mechanisms that control early development. The primary model system is based on the transcriptional regulation of an epidermal keratin gene, XK81A1, in the amphibian (Xenopus laevis) embryo. The cis-regulatory elements of this gene have been mapped using transgenic frog embryos as an assay system. One regulatory site has been precisely located and characterized in detail. A positive transcription factor, KTF-1, binds to this DNA and stimulates the activity of the keratin promoter. Characterization and cloning of KTF-1 are currently being pursued, and other positive and negative regulatory elements in the keratin promoter are being investigated.

In addition, other developmentally interesting genes have been recovered from two subtracted cDNA libraries prepared as part of this program: UVS.1 and UVS.2 (for ultraviolet sensitive) are expressed specifically in cement gland and hatching gland, respectively. These and other genes in this series are markers for dorsoanterior axis formation. DG72 is expressed specifically in the posterior region of the embryo from gastrulation through early organogenesis. This gene is inducible with the mesoderm inducer XTC-MIF, and the predicted protein sequence includes a region similar to the "zinc finger-like" RNA binding domain of retroviral gag proteins as well as an extended acidic helix with a short "leucine zipper". The regulation and function of these genes are subjects for ongoing and future

LABORATORY OF THEORETICAL AND PHYSICAL BIOLOGY (LTPB)

Z01 HD 00040-14	Statistical and Mathematical Studies of Molecular Interactions Peter J. Munson, Ph.D.
Z01 HD 00165-14	Isolation and Characterization of Macromolecular and Cellular Particles Andreas Chrambach, Ph.D.
Z01 HD 00171-13	Electrophoretic Methodology Andreas Chrambach, Ph.D.
Z01 HD 00189-08	Computer Programs for Analysis of Laboratory and Clinical Data David Rodbard, M.D.
Z01 HD 01400-07	Clinical Applications of Stable Isotopes Alfred L. Yergey, Ph.D.
Z01 HD 01401-07	Biological Applications of Thermospray Liquid Chromatography/Mass Spectrometry Alfred L. Yergey, Ph.D.
Z01 HD 01404-06	Characterization of Opioid and Peptide Receptors in Brain and Peripheral Tissues David Rodbard, M.D.
ZO1 HD 01405-05	Computer Programs to Aid Intensive Insulin Therapy for Type-I Diabetes Mellitus David Rodbard, M.D.
ZO1 HD 01406-01	Cell-Cell Communication Between Mammary Epithelium and Connective Tissue Juan C. Calvo, Ph.D.

Laboratory of Theoretical and Physical Biology

The Laboratory of Theoretical and Physical Biology (LTPB) has continued to be highly productive in a multidisciplinary program involving theoretical, statistical, biochemical and physical-chemical approaches to problems relevant to child health and human development. The Section on Theoretical Biology is engaged in the development of computer programs to assist in the management of insulin-dependent diabetes mellitus, development of new algorithms and computer programs to assist in endocrinological research, and laboratory studies of receptors for hormones, drugs, and neurotransmitters. The section on Metabolism and Mass Spectrometry is engaged in studies of calcium metabolism in the neonate, infant, normal children, and during pregnancy and lactation. Further, it is developing and applying the techniques of thermospray liquid chromatography-mass spectroscopy to the study of metabolism of glucose, Vitamin D, cortisol, testosterone, and small peptides. The Section on Macromolecular Analysis is engaged in studies of gel electrophoresis of proteins, nucleic acids, and protein-nucleic acid complexes.

The Section on Theoretical and Physical Biology (**David Rodbard**) have developed a series of computer programs to assist in the management of patients with insulin-dependent diabetes mellitus. We have developed a minimally complex mathematical model to describe the essential features of the pharmacokinetics and pharmacodynamics of subcutaneous insulin. This program allows one to predict the effects of changes of insulin types, regimen, or dosage and the timing and carbohydrate content of meals. This program can be used for education of patients and physicians. After further studies to validate the model, this program may also be used, as part of an expert system, to make recommendations to physicians about patient management. Other programs have been developed and refined, for analysis of data from self-monitoring of blood glucose.

The Unit on Biostatistical Methodology (**Peter Munson**) has developed several new methods and algorithms. The "FLEXIFIT" algorithm, a "universal," self-modelling approach to describe families of dose response curves, has been revised to improve efficiency and speed of computation and to estimate the effective number of degrees of freedom, the root mean square error, standard errors of parameters, and improved methods of hypothesis testing. New methods have been developed, for detection of peaks of episodic hormone secretion, to deconvolute the plasma hormone levels to derive the instantaneous rate of hormone secretion, and to estimate the kinetics of hormone clearance. The limitations of previous methods have been examined. New methods have been developed, to evaluate the coincidence or concordance of episodic events in two hormonal time series. This will facilitate the investigation of co-secretion of hormones (e.g., LH, FSH, LH alpha-subunit, Prolactin, etc.), and of the relationship between input and output of a gland (e.g., LH, testosterone).

The theoretical basis for analysis of hormone receptor systems has been expanded. A new method has been developed, to derive a two-dimensional affinity spectrum for one labeled ligand and 2 unlabeled ligands reacting with any number of classes of binding sites.

Theory has been developed, to provide optimal design of experiments for systems involving one or two classes of binding sites. When more than one binding sites is

present, then a dose response surface for two ligands can give considerably more information than a conventional self- and cross-displacement experiment. A new computer program, "DESIGN," calculates the optimal combinations of ligand concentrations on this surface. This program and experimental strategy has been applied successfully to the characterization of glucocorticoid receptors in hypothalamus (in collaboration with DEB).

In laboratory studies, we have utilized programs DESIGN and LIGAND, to characterize the receptors for phencyclidine (PCP) and sigma ligands (SKF10,047) in membranes prepared from rat and guinea pig brain. We find that the PCP receptor is present in both high and low affinity forms. The high affinity form appears to be stabilized by the presence of L-glutamate and thus appears to be a site in the ion channel allosterically modulated by excitatory amino acid receptors of the NMDA type. We also obtained evidence, that homocysteic acid may be a naturally occurring agonist at this site. Its activity is virtually identical to that of L-glutamate, it shows stereospecificity, and it is appropriately modified by allosteric regulators and antagonists at the NMDA binding site.

In a new development, we are studying cell-cell communication between normal mammary epithelium and connective tissue, using 3T3 fatty fibroblasts and 3T3-L1 preadipocytes in vitro. We find that adipocytes and fibroblasts stimulate the rate of proliferation and alter the morphology of mammary epithelium. Further, the mammary epithelium produces a factor, present in conditioned medium, which results in inhibition of the transformation of 3T3-L1 cells to adipocytes. This can be measured morphologically, by Oil-Red-O staining, or by measurement of triglyceride accumulation. Current studies are underway to characterize this factor (or factors) biochemically.

The Section on Metabolic and Mass Spectrometry (**Alfred L. Yergey**) conducts research on the clinical applications of stable isotopes. Studies are primarily in two areas: 1) Studies of calcium metabolism using thermal ionization mass spectrometry (TIMS); and 2) Studies of thermospray liquid-chromatography mass spectrometry for metabolic, endocrinologic and structural studies.

1) We seek to elucidate metabolic kinetics for calcium in populations that are inaccessible to study with radiotracers, particularly children and women of childbearing age. To date this objective has been met by studies of the kinetics of calcium distribution in both normal children and in children with disease related to changes in calcium metabolism, as well as in normal lactating and nonlactating women.

The mass of calcium (Ca) in the rapidly exchanging internal pool (MPCa) has been determined from the zero time intercept of a sum of exponentials fit to the data obtained from the time dependent dilution of an intravenously administered tracer. This pool size has been shown to differ substantially from values expected on the basis of considering the pool to be a fixed fraction of body mass. The differences from the expected values in normal children (age range 2 wks - 14 yrs) correlate significantly ($r=0.95$, $p<0.01$, $n=10$) with increase in bone mineral content (mg/cm/wk). This suggests that this pool is an indicator of physiologically active bone mass or bone formation and potentially could be used as a predictor of skeletal growth in children and bone loss in older subjects.

Studies of fractional absorption of dietary calcium in normal adult women have shown excellent agreement between radioactive and stable isotope tracer methodologies. When adjusted for dietary intake, these results show a total absence of age dependence for calcium absorption. In addition, age matched women with osteoporosis absorb calcium

at the same level as normal women but appear to have an increased rate of urinary calcium excretion.

Studies of fractional absorption in premature infants have been expanded to determine endogenous fecal excretion of calcium. This quantity, along with urinary and total fecal calcium excretion allows the estimation of calcium retention. These studies suggest that more calcium is being retained by these infants than had previously been believed. This may affect feeding protocols for these infants.

2) Biological Applications of Thermospray LC/MS: We have developed and applied new methods for analysis of biological materials that require mass spectrometric analysis but which have been difficult or impossible to analyze by other methods because of the thermal lability, involatility or charge state of the molecule.

Cortisol Production Rate (FPR) measurements in normal adults yield an average value of 9.6 $\pm$ 2.6 mg/24 hrs, about 50% lower than the previously generally accepted value, and shows the anticipated circadian variation. The small range of values is consistent with the remarkably low variation observed in mean daily plasma cortisol concentration, [F], of 6 $\pm$ 0.9 μg/dl determined by our isotope dilution methodology. FPR and plasma cortisol values are highly correlated in the normal volunteers, indicating that hormone secretion rather than its metabolism is responsible for the changes in plasma level.

The methodology has been validated in 6 adrenalectomized patients by infusing a known amount of cortisol at known ratio of labelled to unlabelled material over the course of a day. Daily FPR differed by less than 6% from expected. No isotope effect was observed in vivo, i.e., the ratio of labelled to unlabelled material observed in plasma did not differ from that in the infusate. Furthermore, based on in vitro studies, there was no isotope effect seen in solvent extraction.

In 19 patients with surgically proven Cushing's Syndrome (CS), the method showed an increased FPR accompanied by loss of diurnal rhythm of cortisol excretion. Both the 24 hr and 8 PM-12 Midnight FPR allow separation of normal subjects from those with Cushing's Syndrome. In addition, 17-hydroxycorticosteroid (17OH) excretion was seen to correlate better with FPR than did urinary free cortisol (UFC), suggesting that the former is a more sensitive and specific diagnostic test.

FPR was also measured in 33 healthy children ages 8-18. Overall FPR was 9.5 $\pm$ 2.5 mg/24 hr (6.8 mg/m^2/24 hrs.). This is approximately half of the currently accepted value. This may explain why many children with adrenal insufficiency grow poorly when treated with glucocorticoid replacement doses. The replacement dose should be re-evaluated carefully. There were no differences by gender nor did FPR vary with stage of puberty.

The Section on Macromolecular Analysis (**Andreas C. Chrambach**) has turned its attention, to the development of gel electrophoretic methods for obtaining the size, conformation and surface net charge of DNA and DNA-protein interaction products. Four major contributions to that field have been: i) extension of the DNA size range amenable to polyacrylamide gel electrophoresis significantly beyond previous limits, ii) introduction of discontinuous buffer systems into DNA electrophoresis; iii) application of Ferguson plot (log mobility vs. gel concentration) analysis to the determination of the conformational consequences of protein binding to DNA; iv) optimization of electrophoretic parameters for the Ferguson plot analysis of DNA.

i) To date, DNA up to 1 kbp has been routinely analyzed by polyacrylamide gels, while

agarose can be used up to 25 kbp. Using highly crosslinked polyacrylamide we were able to extend the DNA size range applicable to PAGE 10-20 kbp. Compared to agarose, polyacrylamide has the advantage of sharper zones and higher resolution, relative absence of zone perturbations and improved capability with the operations of discontinuous buffer systems. Moreoever, polyacrylamide gel fibers are not oriented by electrophoresis as are agarose fibers, and polyacrylamide has the advantage of not exhibiting electroendosmosis.

ii) Mobility measurement relative to a moving boundary, generated by a discontinuous buffer system, has not hitherto been applied to DNA electrophoresis. Its introduction has resulted in elimination of the substantial labor and imprecision involved in the measurement of absolute electrophoretic mobilities. These benefits made it practical to apply Ferguson plot analysis to DNA.

iii) Ferguson plot analysis is important because it allows one to interpret the electrophoretic band in terms of molecular size and net charge independently of standards. It provides an experimental tool for testing the widely accepted assumption that the migration distance of DNA is indirectly proportional to size, and for detecting conformational alterations, bound proteins and counterion compositions which vitiate that assumption.

Ferguson plot analysis of DNA conformations was applied to determining the size and net charge of two 155 bp DNA fragments with a repressor protein bound either centrally or peripherally. Protein binding in a central location of one of these species was found to give rise to a larger DNA molecule relative to the one which had previously been considered a bent DNA with the larger bending angle (which had been expected to behave in a larger complex if bending had been responsible for its electrophoretic retardation). Peripheral binding was ineffective in producing any conformational differences between the two mutant complexes.

All protein binding was found to result in a diminished surface net charge. The centrally bound complex exhibited a decreased surface net charge relative to either free DNA or the peripherally bound complex. These findings are based on another new method devised in the Section, viz. the measurement of mobility in copolymers of polyacrylamide with agarose. This technique, by providing mobility data in a non- or minimally sieving medium overcomes the ambiguity and error involved in the determination of free mobility (surface net charge) by extrapolation of linear Ferguson plots to 0 gel concentration.

iv) Work was begun to optimize the conditions of Ferguson plot analysis of DNA. Decrease in the operative ionic strength from the conventional 0.04 M or higher to 0.075 M resulted in considerable time saving with no sacrifice in resolution. Systematic studies have optimized temperature, field strength and the DNA: ethidium bromide concentration ratio.

2) The Section has continued its work aiming at a gel electrophoresis apparatus optimized for "Ferguson plot analysis", i.e., simultaneous runs at several gel concentrations, at the nanogram load level, under avoidance of gel dehydration ("submarine") and adaptable to "discontinuous" buffer systems. For the first time, a prototype apparatus with these properties has been constructed.

3) The Section has developed a computer method for evaluating polydisperse mixtures of subcellular-sized particles with regard to size and surface net charge. Such evaluation is of importance in the production of vaccines made by conjugation of

bacterial polysaccharides with proteins. We have developed a program for the Macintosh II computer capable of providing the relative abundance of each component species together with its size and net charge, using an imput of a two-dimensional densitometric pattern.

4) Previous work in the Section on the elaboration of an operationally simplified technique of Immobiline electrofocusing was continued in 2 directions: First, the Peltier-cooled apparatus used for that purpose was adapted to allow for focusing on immobilized pH gradients under a layer of silicone oil. This prevents water exudation and dehydration problems, and allows for the long duration of focusing required to obtain gel patterns which remain constant and reproducible between experiments and laboratories. Secondly, conditions for diffusion blotting of Immobiline gels were found which circumvented the electroendosmotic problems of electrotransfer of zones from such gels. The method has been applied successfully to the determination of the carbohydrate composition of an 18-component acid phosphatase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00040-14 LTPB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Statistical and Mathematical Studies of Molecular Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. Munson	Mathematical Statistician	LTPB, NICHD
Others:	D. Rodbard	Head	LTPB, NICHD
	K. Chen	Visiting Associate	LTPB, NICHD
	E. Rovati	Visiting Fellow	LTPB, NICHD
	R. Jernigan	Volunteer Researcher	LTPB, NICHD
	M. Jaffe	Volunteer Researcher	LTPB, NICHD

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Theoretical and Physical Biology

SECTION
Section on Theoretical Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.25	2.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Implementation and refinement of the algorithm underlying the FLEXIFIT method was completed, resulting in two completed FORTRAN programs, FLEXIFIT v2.1 and FLEXIFIT v3.1. This method combines the advantages of empirical, nonparametric methods with those of traditional parametric modeling approaches. It seeks to characterize the common shape of a family of curves, and then to assess differences between curves via four linear scaling parameters. A substantial theoretical difficulty had been finding an estimate for the effective "degrees of freedom" for the residual sum of squares after estimating the shape and four additional parameters. The theoretical solution involves calculation of the trace of a matrix. An efficient means for this calculation has now been incorporated into the FLEXIFIT program. Further, the new algorithm for minimization of a penalized sum of squares has been implemented. Tests show it to be much more stable than previous algorithms to changes in initial estimates.

A second phase of the study of optimal design for ligand binding has been completed, with a compilation of designs for two-ligand experiments. Unlike the earlier designs for a single ligand, no universal rules or patterns emerged to describe such designs. Application of the sequential refinement of designs technique has been accomplished for a glucocorticoid receptor assay, resulting in an assay design requiring only a single animal rather than 15.

New investigations were begun into the problems of analysis of hormone pulsatile data, specifically finding optimal estimates for the number of pulses in a convolved binomial-gaussian process. Substantial progress was made in finding algorithms for calculating the two-dimensional discrete and continuous affinity distribution from a family of binding curves. A theoretical link between these two problems was found utilizing a new algorithm for smooth, constrained numerical deconvolution.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00165-14 LTPB

PERIOD COVERED
October 1, 1988 to September 30, 1989.

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Isolation and Characterization of Macromolecular and Cellular Particles

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Chrambach	Head	LTPB, NICHD
Others:	L. Orban	Visiting Fellow	LTPB, NICHD
	A. Kubicz	Courtesy Associate	LTPB, NICHD
	S. Ben-Or	Special Volunteer	LTPB, NICHD

COOPERATING UNITS *(if any)*
Laboratory of Molecular Biology, NCI (C. Zwieb and S. L. Adhya); Division of Cancer Etiology, Laboratory of Tumor Cell Biology, NCI (J. Gershoni).

LAB/BRANCH
Laboratory of Theoretical and Physical Biology

SECTION
Section on Macromolecular Analysis

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1) Electrophoretic retardation due to binding of a repressor protein at a central location of a DNA restriction fragment, in two mutants, has been interpreted previously in terms of different degrees of DNA bending induced by the protein. This interpretation of retardation at a single gel concentration has been critically reinvestigated, using the rate of change of mobility with gel concentration as a measure of the size and conformational state of DNA. It was shown that the DNA previously thought of as "more highly bent" is in fact the larger species, suggesting that the conformational effect of protein binding in this case is to stretch the molecule rather than to condense it. In agreement with previous reports, however, no conformational differences of peripheral protein binding on DNA was found in the same two mutants.

2) The isoelectric zone composition of 18 molecular forms of acid phosphatases from frog liver was determined by isoelectric focusing on a immobilized pH gradient gel. Focusing to the steady-state (52,000 V-h) was achieved on a Peltier cooled gel support plate, with the gel submerged under silicone oil to prevent water exudation.

3) A study was conducted to identify molybdenum (Mo)-binding proteins associated with glucocorticoid receptor which act as inhibitors of receptor transformation. The possibility that Mo-bound proteins, separated from the receptor by isoelectric focusing on Sephadex, are identical to heat shock protein 90 was explored.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00171-13 LTPB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Electrophoretic Methodology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Chrambach	Head	LTPB, NICHD
Others:	D. Tietz	Visiting Scientist	LTPB, NICHD
	L. Orban	Visiting Fellow	LTPB, NICHD
	J. Pospichal	Visiting Fellow	LTPB, NICHD
	A. Kubicz	Courtesy Fellow	LTPB, NICHD
	J. S. Fawcett	Consultant	LTPB, NICHD

COOPERATING UNITS *(if any)*

Biomedical Engineering and Instrumentation Branch, NIH (A. Al-Droubi, M. Unser)

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Macromolecular Analysis

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	2.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

(1) A method for determination of the free mobility (surface net charge) of macromolecules based on the Ferguson plot in agarose-polyacrylamide copolymers was developed. The method, for the first time, avoids the error due to the assumption of Ferguson plot linearity in the lowest gel concentration range (0 to 3.5% total acrylamide (T)). It thereby recaptures the key advantage of gel electrophoresis over chromatography in the physical characterization of macromolecules, i.e., the simultaneous evaluation of surface net charge and size of macromolecules. (2) A discontinuous (moving boundary electrophoresis) buffer system for DNA fragments in the size range of 8 to 23,000 bp was developed which by replacing the characteristic absolute mobilities by relative ones is capable of reducing the error in zone identification. (3) A method for polyacrylamide gel electrophoresis of DNA fragments, 0.3 to 23 kbp in size, using gel 15% crosslinked with DATD was developed which yields superior resolution compared to agarose gels conventionally used in that size range. (4) The dependence of the electrophoretic mobility of DNA fragments on ethidium bromide concentration used in prelabeling, on temperature and ionic strength was determined. (5) A method for evaluating polydisperse gel patterns yielding size, net charge and relative concentration at each point in the pattern was developed. The method advances one previously developed in application to semisynthetic protein conjugates with bacterial coat carbohydrates of importance in vaccine production, by being able to assign relative concentrations to the components characterized by size and charge. (6) A gel electrophoresis apparatus for thin-layer, horizontal gel strips submerged under inert solvents while applicable to discontinuous buffer systems was constructed. The apparatus is the first to combine "stacking" of large sample volumes, "submarine" operation which prevents gel dehydration during electrophoresis, and reduction of load from the microgram to the nanogram level. (7) A technique of electrofocusing in immobilized pH gradients, using a gel submerged under silicone oil and thermostated by Peltier cells was developed. The method prevents water exudates on the gel surface and dehydration without loss of efficient temperature control, and therefore allows for prolonged electrofocusing frequently required for attaining the isoelectric endpoint of a protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00189-08 LTPB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Computer Programs for Analysis of Laboratory and Clinical Data

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	D. Rodbard	Head	LTPB, NICHD
Others:	P. Munson	Mathematical Statistician	LTPB, NICHD
	A. Genazzani	Visiting Fellow	LTPB, NICHD
	V. Guardabasso	Institute of Pharmacology "Mario Negri," and "Negri Sud"	LTPB, NICHD
	K. Oerter	Medical Staff Fellow	LTPB, NICHD

COOPERATING UNITS *(if any)*
University of Virginia School of Medicine (J. Veldhuis); Consorcio Negri Sud, Chieti, Italy (V. Guardabasso); University of Modena, Modena, Italy (A. Genazzani)

LAB/BRANCH
Laboratory of Theoretical and Physical Biology

SECTION
Section on Theoretical Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have developed improved methods for detection and characterization of episodic hormone secretion, and to estimate the instantaneous rate of hormone secretion using deconvolution analysis. These methods have been applied to several clinical investigations of the dynamics of LH, FSH, prolactin, ACTH, cortisol, and beta-endorphin.

Several new methods and algorithms have been developed, to permit detection of peaks of hormone release, and to perform "deconvolution" to estimate the instantaneous secretory rate. These new algorithms provide simplicity, speed of computation, and an enhanced basis in statistical theory.

New methods have been developed to evaluate coincidence of pulses in two hormonal time series. These include the concept of "specific concordance" as a function of threshold levels and adjustable lag times, and also uses the Kappa statistic. Program DETECT is being extensively revised to improve speed and user-friendliness.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01400-07 LTPB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Applications of Stable Isotopes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.L. Yergey	Head	LTPB, NICHD
Others:	N. Vieira	Biologist (Tech.)	LTPB, NICHD
	S. Abrams	NRSA	LTPB, NICHD

COOPERATING UNITS *(if any)* Lab. Math. Biol., NCI (D. Covell); U. Tenn., (R. Goans); Dept. Ped. U. MO Med. Sch., Columbia, MO (L. Hillman); Dept. Endoc., Mayo Clinic (R. Eastell); Cin. Child Hosp. (B. Specker); USDA, Beltsville, MD (C. Veillon, P. Moser); Dept. of Nutr., U.Conn. Storrs (L. Allen); Children's Hosp.NMC (A.Fletcher)

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Metabolic and Mass Spectroscopy

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A. Continuing work using thermal ionization mass spectrometric (TIMS) analysis of calcium stable isotopic tracers for the measurement of fractional absorption and endogenous fecal excretion from diet and the kinetics of whole body distribution have led to a number of clinically significant findings. 1) The mass of calcium in the rapidly exchanging internal pool (MPCa) has been determined from the intercept of the curve showing dilution of an intravenously administered tracer. This pool size has been shown to differ substantially from values expected on the basis of considering the pool to be a fixed fraction of body mass. These differences are highly correlated with incremental bone growth as measured by changes in bone mineral content (mg/cm/wk). This suggests that the pool is an indicator of physiologically active bone mass or bone formation. 2) The mean residence of calcium in the body, a measure of total body turnover, has been shown to relate directly to skeletal mass. This relationship has been shown to hold in normal humans over an age range of 2 wks - 45 yrs. 3) Studies of fractional absorption of dietary calcium in normal adult women have shown excellent agreement between radioactive and stable isotope tracer methodologies. 4) Endogenous fecal excretion of calcium may be a much smaller fraction of dietary calcium in infants than previously believed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01401-07 LTPB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biological Applications of Thermospray Liquid Chromatography/Mass Spectrometry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Yergey	Head	LTPB, NICHD
Others:	N. Esteban	Visiting Associate	LTPB, NICHD
	D. Vicchio	IRTA	LTPB, NICHD

COOPERATING UNITS *(if any)*
Children's Hospital Nat'l Med. Center (T. Silver); U. Conn. (L. Allen); DEB, NICHD (L. Loriaux and T. Loughlin, F. Casorla, K. Karalis, B. Linder, J. Zwadzki); NCI P-Navy MOB (J. Mulshine, T. Treston).

LAB/BRANCH
Laboratory of Theoretical and Physical Biology

SECTION
Section on Metabolic Analysis and Mass Spectrometry

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

(1) a) Continued study of daily cortisol production rates (FPR) in patients and normal volunteers by stable isotope dilution liquid chromatography/mass spectrometry (ID-LC/MS) has revealed a number of significant findings. The value of FPR in normal volunteers (n = 11) is 9.6± 2.6 mg/24 hrs (RSD = 27%) is both lower in absolute value and has a smaller range than determinations by other methods. The methodology has been validated in 6 adrenalectomized patients by administering a known amount of cortisol at known ratio of labelled to unlabelled material over the course of a day. Daily FPR differed by less than 6% from expected. No isotope effect was observed in vivo, nor, in separate extraction studies, in vitro. b) In 19 patients with surgically proven Cushing's Syndrome (CS), the method showed an increased FPR with loss of diurnal rhythm. 17-hydroxysteroid excretion correlated better with FPR than did urinary free cortisol. c) FPR was measured in 33 healthy children ages 8-18. Overall FPR was 9.5±2.5 mg/24 hrs (6.8 mg/m2/24 hrs.). This is approximately half of the currently accepted value. There were no differences by gender, nor did FPR vary with pubertal stage.

(2) Several alpha-carboxyamidated peptides that serve as models for peptide autocrine growth factors of small cell lung carcinoma have been characterized using a combination of peptidyl amino acid hydrolase and thermospray LC/MS. Preliminary work suggests that there is some ability to obtain sequence information from this approach.

(3) Measurement of plasma levels of 25-OH vitamin D3 (25OHD) in 5 normal subjects using ID-LC/MS and a trideutero 25-OHD internal standard gave 26 mg/ml, a value that does not differ from that determined by other methods.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01404-6 LTPB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Opioid and Peptide Receptors in Brain and Peripheral Tissues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Rodbard	Head	LTPB, NICHD
Others:	S. Schwarz	Visiting Scientist	LTPB, NICHD
	G. Z. Zhou	Visiting Associate	LTPB, NICHD
	A. Katki	Chemist	LTPB, NICHD
	H. Xu	Courtesy Associate	LTPB, NICHD

COOPERATING UNITS *(if any)*

Institute for General & Experimental Pathology, University of Innsbruck, Innsbruck, Austria (S. Schwarz)

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Theoretical Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.5	0.25	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have used quantitative ligand binding studies to characterize complex, heterogenous receptors for hormones and neurotransmitters in brain and other tissues. We have evaluated the hypothesis, that homocysteic acid (HCA) is a naturally occurring neurotransmitter for the N-methyl-D-Aspartate (NMDA) type of the glutamate receptor. The NMDA receptor is allosterically coupled to an ion channel, to which the drug MK-801 binds with high affinity and specificity. We examined the binding of [3H]-MK-801 to brain membranes which were thoroughly washed to remove endogenous amino acids and other small ligands. Repletion of L-glutamate, or L-homocysteic acid, resulted in parallel dose response curves for [3H]-MK-801 binding. This effect was potentiated by low concentrations of glycine, a known allosteric modulator of this system. More than 20 other amino acids were ineffective, demonstrating the specificity of L-HCA. A moderate degree of stereoselectivity was demonstrated (L>DL>D). HCA appears to be selective for the NMDA receptor, and not effective at the kainic acid or quisqualate receptors. Together with data (e.g., electrophysiological) from other laboratories, these studies support the hypothesis that L-HCA may be an endogenous neurotransmitter at excitatory amino acid receptors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01405-05 LTPB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Computer Programs to Aid Intensive Insulin Therapy for Type-I Diabetes Mellitus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Rodbard	Head	LTPB, NICHD
Others:	M. Berger	Volunteer Researcher	LTPB, NICHD
	P.J. Munson	Statistician	LTPB, NICHD

COOPERATING UNITS *(if any)*
Yale University School of Medicine (M. Berger); Kantonsspital Basel, Basel Switzerland (M.Berger); Medical Center of Delaware, Wilmington, Delaware (G.S. DeCherney).

LAB/BRANCH
Laboratory of Theoretical and Physical Biology

SECTION
Section on Theoretical Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have developed a computer program for the simulation of plasma insulin and glucose dynamics after subcutaneous injection of insulin. The program incorporates a pharmacokinetic model to calculate the time courses of plasma insulin for various combinations of popular preparations (Regular, NPH, Lente, Ultralente). Utilizing a pharmacodynamic model describing the dependence of glucose dynamics on plasma insulin and glucose levels, the program can predict the expected time course of plasma glucose in response to a change in carbohydrate intake, insulin dosage, timing or regimen. A set of typical parameters has been obtained by analysis of data from the literature. Several computer simulations have been generated to evaluate the effect on a 24 hour insulin and glucose profile of systematically changing insulin regimen, dose, timing of meals or timing of preprandial insulin adminstration. The program can be used to explore on a theoretical basis the impact of various factors associated with glycemic control in IDDM. As an educational tool the program provides a realistic environment for demonstration of the combined or isolated effects of insulin and diet on glycemia. Further, we have developed programs for analysis of blood glucose values obtained by persons performing self-monitoring of blood glucose. These programs provide several novel features, including an animated computer "movie" showing how the 24 hour glucose profile changes as a function of time.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01406-01 LTPB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cell-Cell Communication Between Mammary Epithelium and Connective Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.C. Calvo	Visiting Scientist	LTPB, NICHD
Others:	D. Rodbard	Head	LTPB, NICHD
	A. Katki	Chemist	LTPB, NICHD
	S. Chernick	Consultant	LTPB, NICHD

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Theoretical and Physical Biology

SECTION
Section on Theoretical Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.75	1.25	0.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The orderly development of the breast during thelarche, pregnancy and lactation indicates a coordinated response of multiple cell types. The mammary gland provides an excellent experimental model for the study of the interactions between various cell types, between cells and extracellular matrix and between cells and the hormonal environment. The complexity of its cellular composition, the well defined hormonally controlled cycle and the specificity of its final response (lactation) present a unique possibility for endocrinological as well as developmental biological studies. Moreover, understanding of factors involved in normal mammary gland growth and development should provide insights into the pathophysiology of diseases of the breast, both benign and malignant.

We are studying the bidirectional interactions between mammary epithelium, adipose and connective tissues. We have demonstrated that material secreted from mammary epithelial cells inhibits differentiation of 3T3-L1 pre-adipocytes into mature adipocytes. We have demonstrated this inhibition by direct co-culture of both cellular types, by use of conditioned medium from mammary epithelial cells, and by co-culture with a physical separation of the cell types. Conditioned medium from pre-adipocytes stimulates growth of mammary epithelium (as measured by thymidine incorporation) and stimulates the triglyceride content of 3T3-L1 cells. These results support the hypothesis that bidirectional communication occurs between these cell types. Further experiments are in progress to characterize the nature of the biochemical factor(s) and mechanisms involved.

OFFICE OF THE SCIENTIFIC DIRECTOR (OSD)

Z01 HD 00093-15 — Mechanism of Action of Nerve Growth Factor
Gordon Guroff, Ph.D.

Z01 HD 01500-07 — Cellular DNA Replication, Mutagenesis, and Repair: Studies using Virus and Plasmid Vector Probes
Arthur S. Levine, M.D.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00093-15 OSD

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanism of Action of Nerve Growth Factor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: G. Guroff Head OSD, NICHD

Others: See Attached

COOPERATING UNITS *(if any)*
Tokyo Research Laboratories, Kyowa Hakko Kogyo, Tokyo, Japan (Y. Matsuda)
Institute of Protein Research, Academy of Sciences of the USSR, Moscow Region (A. Ryazanov)
Laboratory of Cell Biology, National Institute of Mental Health (D. Rausch)
Laboratory of Biochemistry, National Heart, Lung, and Blood Institute (S. Rhee)

LAB/BRANCH
Office of the Scientific Director

SECTION
Section on Growth Factors

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda Maryland, 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10.25	9.0	1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Nerve growth factor (NGF) is required for the survival and development of sympathetic, sensory, and certain central nervous system neurons. It binds to receptors on these neurons, initiates a chain of intracellular events, and controls the expression of specific genes. The cascade of intracellular events mediating its actions is becoming clear. Much of this information has been gained through a study of the NGF-responsive cell line PC12. We have found that the binding of NGF to its receptor is followed rapidly by increases in cAMP concentration, phosphoinositide metabolism, and arachidonic acid release. These changes in second messenger levels lead to alterations in protein kinases in the cell and to changes in the phosphorylation of key proteins. Among these are elongation factor 2 and the S6 protein of the ribosomes, and a nuclear protein (SMP) perhaps involved in regulating the transcription of specific genes. We have developed cell-free systems for the phosphorylation of these three proteins, which are being used to dissect the mechanisms by which the phosphorylations are altered. The work has suggested that there are a number of parallel phosphorylation cascades emanating from the receptor. A major effort is to find that single reaction coupling the receptor to these cascades. The search for this reaction is being conducted using a new tool, K-252a, developed in this laboratory. K-252a is a kinase inhibitor specific for the effects of NGF on PC12 cells. There is evidence indicating that the protein products of the protooncogenes src and ras participate in the intracellular chain of events. Using a PC12 line infected with a virus carrying the src gene, we have shown that the differentiation seen has different characteristics than that produced by NGF, suggesting that src is not involved in NGF-induced differentiation. Comparable studies with a ras-infected strain are now in progress. Other studies have focused on the expression of specific gene products that underlie NGF-induced differentiation. We have found that the expression of the cell recognition molecule Ng-CAM, increased by NGF, is prevented by glucocorticoids. We have shown that glucocorticoids cause a down-regulation of the NGF receptor. We have seen that NGF causes a decrease in the activity of calpain, a calcium-activated intracellular protease implicated in the metabolism of structural proteins. This decrease is caused by an increase in the activity of endogenous calpain inhibitor, calpastatin. These changes may be directly involved in the generation of neurites. We are exploring the molecular basis of the NGF-induced down-regulation of receptors for epidermal growth factor (EGF), a mitogen for these cells. The decrease in mitogen receptors could be due to an NGF-induced alteration in their phosphorylation state. The mechanism by which NGF changes the ability of the cell to respond to EGF, and to other mitogens, such as insulin-like growth factor II or bombesin, may yield an important insight into the control of differentiation and cell division.

Others:	G. Dickens	Biological Laboratory Technician	OSD, NICHD
	B. Rudkin	Staff Fellow	OSD, NICHD
	B. Nikodijevic	Visiting Scientist	OSD, NICHD
	S. Koizumi	Visiting Fellow	OSD, NICHD
	M. Tocco	Visiting Fellow	OSD, NICHD
	T. Mutoh	Visiting Fellow	OSD, NICHD
	D. Fink	PRAT	OSD, NICHD
	R. Vorce	IRTA	OSD, NICHD
	S. Doll	Biotechnology Fellow	OSD, NICHD
	M. Oshima	Adjunct Scientist (Courtesy)	OSD, NICHD
	K. Fujita	Adjunct Scientist	OSD, NICHD
	M. Sutphin	Federal Junior Fellow	OSD, NICHD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01500-07 OSD

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cellular DNA Replication, Mutagenesis, and Repair: Studies using Virus and Plasmid Vector Probes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.S. Levine	Head			OSD, NICHD
Others:	C.T. Patch	Sr. Investigator	M. Abramic	Visiting Fellow	OSD, NICHD
	K. Dixon	Sr. Investigator	J. Carr	Biotech. Fellow	OSD, NICHD
	M. Protic	Visiting Assoc.	M. Carty	Visiting Fellow	OSD, NICHD
	J.M. Hauser	Microbiologist	E. Kajiwara	Visiting Fellow	OSD, NICHD
	M. Carbone	Exchange Scientist	M. Zernik-Kobak	Exchange Scientist	OSD, NICHD

COOPERATING UNITS *(if any)* LIP, NIAID (A.M. Lewis, Jr.); LDP, NICHD (D. Nebert); LDMI, NICHD (S. Hirschfeld & K. Ozato)

LAB/BRANCH

Office of the Scientific Director

SECTION

Section on Viruses and Cellular Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10	9	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mutations are the underlying cause of most inherited diseases, many developmental abnormalities, and in all likelihood, most tumors. We are studying the process of mutagenesis using DNA virus-based shuttle vectors as probes to investigate the molecular mechanisms by which agents that damage DNA induce mutations in mammalian cells. Through use of such vectors, we have extensively characterized the types of mutations that occur in mammalian cells either spontaneously or in response to DNA damage. Analysis of the sequence specificity of these mutations has led to models which explain how the mammalian DNA polymerase introduces errors during DNA synthesis, causing mutations. Studies with the vector in an in vitro DNA replication system indicate that cellular factors, in addition to DNA polymerase, appear to influence replication fidelity. Further studies with this system should allow a characterization of the cellular factors that determine both spontaneous and induced mutagenesis in mammalian cells.

DNA repair processes play an important role in protecting cells against the mutagenic and carcinogenic action of toxic agents. With the use of virus-based expression vectors in vivo and in vitro, we are studying the molecular mechanisms by which DNA lesions are repaired in mammalian cells. Our results indicate that repair functions are enhanced after DNA damage. Enhanced repair is accompanied by the induction of a lesion-specific DNA binding protein, which suggests its direct involvement in DNA repair.

Understanding the mechanisms of regulation of cellular proliferation and differentiation (which in turn relate to DNA replication) is basic to understanding development of multicellular organisms. These mechanisms can be addressed in transformed as well as in normal cells, since the results of many studies on oncogenes demonstrate that differences between controlled and uncontrolled cell growth are--at the molecular level--subtle. In the past, we have focused on an experimental rodent model for studies of DNA virus oncogenicity, and have illuminated a number of complex interactions between viral genes, cellular genes, and the host's immune system. Currently, we have undertaken studies of a subset of human and hamster tumors which may serve as models for the molecular analysis of interactions between oncogenes and anti-oncogenes.

PREVENTION RESEARCH PROGRAM

BIOMETRY BRANCH (BB)

Z01 HD 00801-14	Studies Based on the Medical Birth Registries of Norway and Sweden Howard J. Hoffman, M.A.
Z01 HD 00802-14	Studies of Linked Live Births-Infant Deaths and Fetal Deaths from U.S. States Howard J. Hoffman, M.A.
Z01 HD 00803-05	Analysis of Sudden Infant Death Syndrome (SIDS) Risk Factors Howard J. Hoffman, M.A.
Z01 HD 00813-08	Biostatistical Methods for the Analysis of Laboratory Research Studies George F. Reed, Ph.D.
Z01 HD 00818-08	Research in Developing Nonparametric Methods for Biomedical Applications George F. Reed, Ph.D.
Z01 HD 00841-08	Methods for Comparing and Analyzing Data from Several Complex Surveys Barry I. Graubard, M.A.
Z01 HD 00842-07	Development of Statistical Methods to Analyze Cluster Samples Barry I. Graubard, M.A.
Z01 HD 00850-13	Randomized, Controlled Study of Phototherapy for Neonatal Hyperbilirubinemia Dolores A. Bryla, M.P.H.
Z01 HD 00853-05	Design and Analysis of a Clinical Trial of Vi Polysaccharide Vaccine Dolores A. Bryla, M.P.H.
Z01 HD 00854-05	Analysis of MCH Data from the National Longitudinal Youth Survey Dolores A. Bryla, M.P.H.
Z01 HD 00860-09	Analysis of Biomedical Time Series Data Howard J. Hoffman, M.A.
Z01 HD 00861-07	Assessment of In-Utero Fetal Growth Patterns in Relation to Outcome at Birth Howard J. Hoffman, M.A.

Z01 HD 00871-04	Clinical Trial of New Drug Therapy for Cystinosis Howard J. Hoffman, M.A.
Z01 HD 00872-04	Factors Associated with Premature Births: Missouri Follow-back Survey Howard J. Hoffman, M.A.
ZO1 HD 00874-02	Research on Racial Differences in Pediatric Measures of Gestational Age George F. Reed, Ph.D.
ZO1 HD 00875-01	National Maternal and Infant Health Survey (NMIHS) Howard J. Hoffman, M.A.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00801-14 BB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies Based on the Medical Birth Registries of Norway and Sweden

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H.J. Hoffman	Special Ass't for Infant Mortality	PRP, NICHD
Other:	A.A. Herman	Visiting Scientist	EB, PRP, NICHD

COOPERATING UNITS *(if any)* Inst. of Hygiene & Soc. Med. & Dept. of OB/GYN, Univ. of Bergen, Norway (P. Bergsjø and L. Irgens); Dept. of Community Medicine, Univ. of Trondheim and Nat'l Inst. of Public Health, Oslo, Norway (L. Bakketeig, A. Arntzen); Dept. of OB/GYN and Social Med., Uppsala Univ. (G. Lindmark, S. Cnagtingius, O. Meirik).

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.1	.1	.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These studies have focused on: (1) the relation of the quality of medical care to the risk of perinatal death in Norway and Sweden, (2) the tendency to repeat similar birth weight and gestational age in subsequent pregnancy outcomes to the same mothers, (3) perinatal mortality in relation to order of birth and size of sibship, (4) epidemiologic risk factors for preterm birth, (5) epidemiologic risk factors for small-for-gestational age births, (6) contribution of multiple births to perinatal mortality rates, (7) size at birth as measured by head circumference, crown-heel length, and birth weight in relation to gestational age and perinatal mortality.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00802-14 BB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Linked Live Births-Infant Deaths and Fetal Deaths from U.S. States

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H.J. Hoffman	Special Ass't for Infant Mortality	PRP, NICHD
Others:	G.F. Reed	Mathematical Statistician	BB, PRP, NICHD
	M.H. Malloy	Research Medical Officer	EB, PRP, NICHD

COOPERATING UNITS (if any) EB, PRP, NICHD (G.G. Rhoads, M.D. Overpeck); EB, BRAP, NIEHS (A. J. Wilcox); Departments of Health in the following states: Michigan, Missouri, New York State, North Carolina, and Utah; Office of International Statistics, NCHS (R. Hartford).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.2	.2	.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives are to assemble a multi-state data file of infant deaths in which prior linkage with birth certificate information has been performed. Similar information regarding fetal deaths, based on reports filed for fetuses of at least 20 weeks gestation, will also be studied. The studies to be done on the data set include associations between infant and fetal mortality with the standard information on birth certificates (e.g., birth weight, gestational age, maternal age, race, parity, etc.). The information on fetal or infant death records includes immediate and underlying cause-of-death categories corresponding to the International Classification of Diseases (ICD), based on either the eighth or ninth revision of the ICD codes. Some additional data are available from selected states regarding: smoking during pregnancy, maternal prepregnant weight and height, weight-gain during pregnancy, occupation of parents, and the levels of obstetric and pediatric care available to mother and infant.

Several research contracts have been jointly funded by NICHD and NIEHS to provide data from selected U.S. States (listed above) to compare with data from other developed countries (Australia, Japan, Norway and Scotland) for the time period, 1980-84. This study is entitled: Multinational Comparisons of Birth Weight-Specific Perinatal Mortality Rates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00803-05 BB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Analysis of Sudden Infant Death Syndrome (SIDS) Risk Factors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: H.J. Hoffman Special Ass't for Infant Mortality PRP, NICHD

COOPERATING UNITS (if any) U. of MD (D. Denman); U. Wash., (D. Peterson, G. van Belle); Loyola U. (J. Goldberg); UCLA (R. Harper, J. Kraus); Columbia U. (J. Parker, E. Krongrad); N.Y. State Hlth Dept. (S. Standfast); U. Mo. (L. Hillman); U. London, U.K. (D. Southall); U. Miami (M. Dapena); U. NM (P. McFeeley); AFIP (T. Stocker).

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.4	.3	.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The NICHD SIDS Cooperative Epidemiological Study was designed to enable identification of risk factors which could differentiate SIDS infants from non-SIDS infants. The design is that of a multicenter, population-based, case-control study with a sample of 838 SIDS cases (800 singleton and 38 multiple birth SIDS cases) ascertained under a common necropsy protocol. There were 1,600 matched living singleton control infants and 40 co-multiple birth control infants recruited into the study. It is the largest detailed epidemiological study of SIDS ever undertaken. Data were collected for babies who died over a 15-month period from October, 1978 through December, 1979. Every infant death was autopsied in accordance with a common necropsy protocol developed specifically for the study. Twenty-six different slides of tissues were preserved for detailed examination by a panel of three SIDS pathology experts. Under an Inter Agency Agreement with the Armed Forces Institute of Pathology (AFIP), technical support is being provided for the preparation of a SIDS Histopathology Atlas and "study sets" to be used for the education of practicing forensic pathologists or pathology students. Also, the possible association between elevated fetal hemoglobin (Hb F) levels and SIDS is being investigated in a study begun during the past year.

In another SIDS risk factor study, techniques of time series analysis are being used to examine potential abnormalities in the development of neuro-physiological and cardio-respiratory control mechanisms in the first three months of life. The study materials consist of computerized data sets from long-term electrophysiological recordings of infants from three earlier SIDS research studies. Comparisons have been made among the following groups of infants: subsequent siblings of SIDS infants, "near-miss" infants, twins, matched controls, and infants who later died of SIDS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00813-08 BB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 charecters or less. Title must fit on one line between the borders.)
Biostatistical Methods for the Analysis of Laboratory Research Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G. F. Reed Special Ass't for Infant Mortality PRP, NICHD

COOPERATING UNITS (if any)
BS, EBB, DMID, NIAID (G. Reed); CPD, CC, NICHD (R. Elin and M. Ruddell); IRP, NIAID (D. Alling and S. Banks); Dept. of Statistics, Harvard U. (D. Hoaglin); Univ. of MD (D. Denman).

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
.3	.2	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Research in design and analysis problems arising from laboratory studies on: (1) dose-response relationships, (2) bioassay and potency estimation, (3) time to event, life table analyses, and (4) other investigations of the effects of external stimuli.

In addition to work on techniques for estimating tolerance limits for chemical residue depletion in animals, a major effort in this research area has arisen in the analysis of data from the Clinical Center's Normal Range Study. This study has resulted in the collection of a large number of biochemical and clinical measurements taken serially for 2½ years from "normal" volunteers. The object of the analysis is to characterize the distribution of each variable in order to determine values that can be considered normal. Some of the statistical techniques to be applied will be exploratory data analysis methods, including graphical techniques and outlier detection, transformation of variables, analysis of variance components, and serial correlation. The results of this project will appear in several published reports of quantitative characterizations with special reference to factors that may affect these distributions, such as smoking, drinking, and eating habits, and other demographic or socio-economic factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00818-08 BB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Research in Developing Nonparametric Methods for Biomedical Applications

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G.F. Reed	Mathematical Statistician	BB, PRP, NICHD
Other:	H.J. Hoffman	Special Ass't for Infant Mortality	PRP, NICHD

COOPERATING UNITS (if any)

University of MD (D. Denman); DBSB, CPR, NICHD (B. Graubard).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.2	.2	.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective is to investigate and develop distribution-free methods in areas of application for which standard parametric techniques are inappropriate or too insensitive to violations of underlying assumptions.

Much of the work of the Branch lends itself to the nonparametric approach. In sample size studies involving analysis of 2x2 tables, the determination of the minimum detectable risk for a given sample size is often required. Although techniques based on asymptotic results for this have been developed within the Branch, they must ultimately be validated by comparison with an exact technique which is based on the theory of randomization testing. This technique has been developed as part of this project.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00841-08 BB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Methods for Comparing and Analyzing Data from Several Complex Surveys

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B.I. Graubard	Mathematical Statistician	DBSB, NICHD
Other:	H.J. Hoffman	Special Ass't for Infant Mortality	PRP, NICHD

COOPERATING UNITS (if any)

Biomathematics Department, School of Medicine, (E. Korn).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.2	.2	.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study will develop statistical methods for the analysis of data from complex sample surveys and test them empirically using the National Health and Nutrition Examination Survey I and II (NHANES). The research concentrates on ways to test hypotheses about the coefficients that derive from multiple linear regression of survey data. Existing methods which use Wald test statistics are compared to newly developed approaches based upon Bonferroni t-statistics and jackknifed Wald statistics. These regression methods will be applied to the NHANES data sets to determine if they can be used to provide ways to analyze the complex relationships of growth and nutrition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00842-07 BB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Statistical Methods to Analyze Cluster Samples

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B.I. Graubard	Mathematical Statistician	DBSB, NICHD
Other:	H.J. Hoffman	Special Ass't for Infant Mortality	PRP, NICHD

COOPERATING UNITS (if any)

BB, PRP, EMS, NCI (M. Gail and T. Fears).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.2	.2	.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This research project will study statistical methods for analyzing categorical data that comes from cluster samples where the observations within each cluster may be correlated and where the observations may be selected with unequal probabilities. In particular, the analysis of cluster samples from population-based case-control studies and cross-sectional and longitudinal health surveys is examined. Research has concentrated on developing modifications to logistic regression and Mantel-Haenzel and Wolf-Haldane procedures that would account for the complex sample design. Computer simulations are used to validate statistical approximations used in the development of modified methods. Preliminary results from this research indicate that the modified methods for analyzing data from cluster samples appropriately take into account the intra-cluster correlation structure and the unequal weighing of the observations. These methods will be useful for analyzing infant feeding studies and repeat pregnancy studies where the family constitutes the cluster.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00850-13 BB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Randomized, Controlled Study of Phototherapy for Neonatal Hyperbilirubinemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D.A. Bryla	Statistician	BB, PRP, NICHD
Others:	H.J. Hoffman	Special Ass't for Infant Mortality	PRP, NICHD
	B.I. Graubard	Mathematical Statistician	DBSB, NICHD

COOPERATING UNITS (if any) Human Learning and Behavior Branch, CRMC, NICHD (P. Scheidt); Intramural Research, Neuroepidemiology Branch, NINCDS (K. Nelson); Intramural Research, Developmental Neurology Branch (D. Hirtz); Computing Sciences Consultant (K. Fetterly).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.6	.4	.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study, which began in 1974, is a cooperative, randomized clinical trial to determine the safety and efficacy of phototherapy for treatment of neonatal hyperbilirubinemia by comparing phototherapy with non-phototherapy infants under specific conditions. Babies were randomized by weight (less than 2,000, 2,000-2,499 and greater than 2,499 grams) to the phototherapy or non-phototherapy groups. Infants, 2,000 grams and above, were admitted to the study when their bilirubin reached levels specified in the study protocol. All infants under 2,000 grams were admitted. Physical, neurological and mental development of these infants were followed through six years of age.

The Biometry Branch served as a data center for this study and was the focal point for receipt of examination forms. The master files for each year's follow-up were edited for keypunch and coding errors and for internal consistency. The Branch is now analyzing the data in cooperation with the principal investigators from the cooperating units. The results of the newborn data were published in a supplement to Pediatrics in February 1985. A manuscript on the follow-up data was submitted for publication to Pediatrics.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00853-05 BB

PERIOD COVERED

October I, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Design and Analysis of a Clinical Trial of Vi Polysaccharide Vaccine

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D.A. Bryla	Statistician	BB, PRP, NICHD
Other:	G.F. Reed	Mathematical Statistician	BB, PRP, NICHD

COOPERATING UNITS (if any)

Office of the Director, NICHD (C.Lowe); Laboratory of Developmental & Molecular Immunity, NICHD (J. Robbins); TEKU Hospital, Nepal (I. Acharya).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.3	.3	.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The study is a cooperative, randomized trial to determine the efficacy of Vi polysaccharide in preventing typhoid fever in Nepal. The Biometry Branch's involvement in this study is to design data collection forms, and assist in the data management and the analysis with the study investigators from NICHD and Nepal.

In March 1986, 6,912 volunteers from five villages in Nepal were randomly vaccinated with either the Vi polysaccharide or pneumonococcal vaccine. These volunteers will be visited every three days for the next two years to verify their health status and to detect any typhoid cases prior to treatment. Blood cultures will be done on anyone with a fever of three days duration. Randomization scheme was broken in August, 1988 and cross-immunization of the volunteers took place in September and October, 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00854-05 BB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of MCH Data from the National Longitudinal Youth Survey

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D.A. Bryla	Statistician	BB, PRP, NICHD
Other:	H.J. Hoffman	Special Ass't for Infant Mortality	PRP, NICHD

COOPERATING UNITS (if any)

Pregnancy and Perinatology Branch, CRMC, NICHD (D. McNellis); Ohio State University (F. Mott).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
.1	.1	.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has as its primary objective to analyze and publish data based on a series of annual interviews of young women (aged 14 to 21 on January 1, 1979) regarding their pregnancy outcome and the first year of life of the child. This survey allows analysis of trends over time in the maternal and child health field of, for example, the use of obstetric technology (diagnostic ultrasound, amniocentesis, etc.), and patterns in breast-feeding. In addition, a wealth of other data have been collected on the youth cohort sample in relation to their employment and work history, military service, educational attainments, etc.

The collection of data on pregnancy outcome and the first year of life of the child began in 1983 and is continuing. With this five year data base, analysis of trends over time in the maternal and child health can be done.

The Biometry Branch has joined in the funding of the data collection effort together with the Demographic and Behavioral Sciences Branch, Center for Population Research, NICHD. The mechanism of support for the field study is through an Inter Agency Agreement with the Department of Labor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00860-09 BB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Biomedical Time Series Data

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: H.J. Hoffman Special Ass't for Infant Mortality PRP, NICHD

COOPERATING UNITS (if any) CI, CP, GRC, NIA (M. Brock); Dept. of Pediatrics, Univ. of South Florida College of Medicine, St. Petersburg, Florida (B. Bercu); Pediatric Nutrition, Mead Johnson Company (J. Hansen); Univ. of Cambridge, England (K. Dalton and G. Breborowicz); Univ. of Alabama in Birmingham (C. Lowery and R. Goldenberg).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
.2	.1	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project are: (1) to characterize developmental patterns from daily measurements of gonadotropins and for estrogens in premenarchial girls and pubescent boys based on radioimmunoassay methods for measuring urinary luteinizing hormone, urinary follicle stimulating hormone, and urinary estradiol, estriol and estrone hormones; (2) gonadotropins in both castrated and intact male monkeys of different ages; (3) growth hormone in normal and precocious pubertal children; (4) to assess circadian and other rhythms in heart rate, temperature and other serial data collected from long-term studies in humans; and (5) to perform analysis of these serial measurements using methods of statistical time series analysis, including autoregressive filtering, auto- and cross-spectrum analysis, and robust smoothing procedures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00861-07 BB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Assessment of In-Utero Fetal Growth Patterns in Relation to Outcome at Birth

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: H.J. Hoffman Special Ass't for Infant Mortality PRP, NICHD

COOPERATING UNITS *(if any)* D. Denman (Univ. of MD); PRP, NICHD (H. Berendes); CRMC, NICHD (D. McNellis); Univ. of Trondheim, Norway (G. Jacobsen, L. Bakketeig); U. of Bergen Norway (P. Bergsjø, T. Evans, T. Markestad); Uppsala Univ., Sweden (G. Lindmark); Bell Commun., Livingston, N.J. (G.W. Reed); U. of AL in Birmingham (R. Goldenberg).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.3	.2	.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project has been expanded to encompass two related research studies. The first study has analyzed data derived from a randomized clinical trial of diagnostic ultrasound use during pregnancy conducted by the team of Norwegian investigators in Trondheim, Norway. The purpose of the analysis is to examine fetal growth patterns using longitudinal measurements throughout pregnancy of: (1) symphyseal-fundal heights; (2) weight gain at each prenatal visit; (3) serial biparietal and abdominal diameter measurements from ultrasound; and (4) maternal hemoglobin level. Regression models have been fit to the serial measurements for each mother. The coefficients of the regressions have been analyzed in relation to various indicators of birth size such as weight, crown-heel length, ponderal index, and birth weight-for-gestational age percentile. Using an analysis of covariance procedure, additional factors (e.g., cigarette smoking, alcohol intake, low maternal prepregnancy weight, etc.) will be tested for significance in modifying intrauterine growth patterns.

In addition to the study described above, a prospective study to determine risk factors for intrauterine growth retardation, or small-for-gestational age birth, was begun in 1984 through the research contract mechanism with both the University of Alabama in Birmingham and University of Trondheim, Norway (in collaboration with the Universities of Bergen and Uppsala). The study protocol includes recruitment of pregnant women before 17 weeks gestation. Those enrolled in the study will be carefully monitored throughout the remainder of their pregnancy. Symmetric and asymmetric forms of intrauterine growth retardation will be assessed prenatally and at delivery. Infants born to the study mothers will have follow-up exams during the first year of life to assess catch-up growth and attainment of early developmental milestones.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00871-04 BB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 charecters or less Title must fit on one line between the borders)*

Clinical Trial of New Drug Therapy for Cystinosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: H.J. Hoffman Special Ass't for Infant Mortality PRP, NICHD

COOPERATING UNITS *(if any)*

BS, EBB, DMID, NIAID (G. Reed); HGB, IRP, NICHD (W. Gahl); Univ. California, San Diego (J.Schneider); Univ. of Michigan Medical School (J. Thoene); Univ. of Texas Health Science Center, Dallas (J. Reisch).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
.3	.3	.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Cysteamine Study provided answers to the question of the drug's efficacy with some inferential difficulty, since cysteamine's unpleasant taste and smell rendered it unpalatable to many patients, who subsequently did not receive effective amounts of the drug. The design of the study itself, with no randomized concurrent control group, obscured effects and required a good deal of reliance on adjustment techniques in the final analysis.

The object of the current study is to improve treatment of cystinosis and determine more of the effects of cysteamine. In the drug development phase of the trial, investigation of a cysteamine analog, phosphocysteamine, revealed that it converts rapidly to cysteamine in the bloodstream, so that the two drugs are effective equivalents. Moreover, since the taste and smell of phosphocysteamine are less obnoxious to some patients, it serves as an alternative treatment that may improve patient compliance. The current study randomizes patients to a low dose of cysteamine (or phosphocysteamine as the patient chooses) or to a high dose; so designed the trial is an optimal vehicle for ascertaining the best course of treatment.

Patient recruitment and treatment is coordinated at contracted study center at the University of California, San Diego. Data center functions are the responsibility of the University of Texas Health Science Center at Dallas. The study will encompass 3-4 years of enrollment and treatment of at least 90 patients. The treatments will be evaluated on the basis of renal function as measured by serum creatinine levels and creatinine clearance, as a surrogate of glomerular filtration rate, at the end of the study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00872-04 BB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Factors Associated with Premature Births: Missouri Follow-back Survey

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H.J. Hoffman	Special Ass't for Infant Mortality	PRP, NICHD
Other:	D.A. Bryla	Statistician	BB, PRP, NICHD

COOPERATING UNITS (if any)

Missouri Department of Health (G. Land, W. Schramm, J. Stockbauer, and V. Pierson).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
.3	.2	.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective is to obtain more accurate information relating to the very low birth weight (VLBW) infant, <1500 grams, than is now available from the United States vital records. This objective will be accomplished by the following: (1) mailing or administering a questionnaire to mothers of VLBW infants, mothers of fetal deaths, and a sample of mothers of LBW infants (1,500-2,499 grams) and normal birth weight infants (≥2,500 grams) in order to obtain and verify information from the prenatal, perinatal, and post-neonatal periods; (2) conducting telephone follow-up interviews on non-respondents and incomplete respondents, and a 10 percent sample of study mothers to obtain and/or verify information on the questionnaires; (3) developing procedures for abstracting information from hospital and physician records, including otherwise unavailable or missing information on morbidity, lifestyle, and socioeconomic indicators of the study subjects; and (4) preparing an edited data tape for NICHD. In addition, mortality and results of follow-up evaluations will be available through the first year of life for this birth cohort.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00874-02 BB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Research on Racial Differences in Pediatric Measures of Gestational Age

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G.F. Reed	Mathematical Statistician	BB, PRP, NICHD
Others:	H.J. Hoffman	Special Ass't for Infant Mortality	PRP, NICHD
	M.A. Klebanoff	Research Medical Officer	EB, PRP, NICHD

COOPERATING UNITS (if any)

Research Triangle Institute (V. Rao).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
.2	.2	.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Dubowitz Examination and its derivative Ballard Examination are instruments for estimating gestational age at the time of birth on the basis of observed physical and neurological maturity. Since racial differences in the distribution of some developmental indices are acknowledged or suspected, it is hypothesized that the current pediatric assessments (i.e., the Dubowitz and Ballard tests), which were constructed and validated on a sample of white babies, may effect a bias in the estimation for other racial groups.

The Vaginal Infections in Prematurity (VIP) Study offers data to test the hypothesis. If it is found that differences do exist, the study will also provide the wherewithal to produce a modified pediatric assessment which is proper for the racial group in question.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00875-01 BB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

National Maternal and Infant Health Survey (NMIHS)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H.J. Hoffman	Special Ass't for Infant Mortality	PRP, NICHD
Other:	D.A. Bryla	Statistician	BB, PRP, NICHD

COOPERATING UNITS *(if any)*

Office of Planning and Evaluation, NICHD (G. Gaines); Followback Survey Branch, National Center for Health Statistics (P. Placek, K. Keppel, G. Simpson, M. Kogan)

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.1	.1	.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects
  - ☐ (a1) Minors
  - ☒ (a2) Interviews
- ☐ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A National Maternal and Infant Health Survey is being conducted by the National Center for Health Statistics with the joint sponsorship of NICHD and several other Public Health Service and federal agencies. The survey consists of three components: a natality survey, which is sampled from certificates of live birth; a fetal mortality survey, which is sampled from reports of fetal death, and an infant mortality survey, which is sampled from infant death certificates. In the summer of 1987, a four-State pretest was conducted for 200 live births, 200 fetal deaths, and 200 infant deaths. Questionnaires were mailed to mothers, hospitals, and prenatal care providers associated with these events. Sponsor agency questions were included in the pretest and then revised as needed for the main survey. Final OMB approval was obtained in the fall of 1988, and the main survey was launched in January 1989. NCHS is monitoring data collection which is being done by the Census Bureau.

EPIDEMIOLOGY BRANCH (EB)

ZO1 HD 00323-09	District of Columbia Perinatal Study Heinz W. Berendes, M.D., M.H.S.
Z01 HD 00325-08	Neural Tube Defects and Folate James L. Mills, M.D., M.S.
Z01 HD 00329-07	Evaluation of an Intervention Trial to Prevent Low Birth Weight in D.C. Mary D. Overpeck, M.P.H.
Z01 HD 00331-06	Diabetes in Early Pregnancy Project (DIEP) James L. Mills, M.D., M.S.
Z01 HD 00334-06	Low Birth Weight Across Generations Mark A. Klebanoff, M.D., M.P.H.
Z01 HD 00340-06	Ethnic Differences in Birth Weight and Length of Gestation Patricia H. Shiono, Ph.D.
Z01 HD 00344-06	Long Term Health Effects of Infant Formulas Deficient in Chloride Michael H. Malloy, M.D., M.S.
ZO1 HD 00346-05	Time Trends in the Incidence of Biliary Atresia Mark A. Klebanoff, M.D., M.P.H.
Z01 HD 00352-04	Studies of Human Immunodeficiency Virus - Related Problems George G. Rhoads, M.D., M.P.H.
Z01 HD 00360-03	A Prospective Study of 1st Trimester Use of Bendectin and Malformations Patricia H. Shiono, Ph.D.
Z01 HD 00361-03	Child Health Supplement to the 1988 National Health Interview Survey Mary D. Overpeck, M.P.H.
Z01 HD 00362-03	Nutritional Aspects of Perinatal Epidemiology in Central America José Villar, M.D.
Z01 HD 00363-02	NICHD Smoking Trial of Pregnant Women (STOP) Leslie C. Cooper, Ph.D.
Z01 HD 00365-02	A Randomized Clinical Trial of Umbilical Artery Catheter Placement Michael H. Malloy, M.D., M.S.
Z01 HD 00366-02	Survey of Pregnancy Outcomes Among Medical Residents Patricia H. Shiono, Ph.D.

Z01 HD 00368-02	Vaginal Delivery of Very Low Birth Weight Infants: Association with Day 1 Deaths Michael H. Malloy, M.D., M.S.
Z01 HD 00369-01	Adverse Perinatal Events and Subsequent Injury-related Death Mark A. Klebanoff, M.D., M.P.H.
Z01 HD 00370-01	Ethnic Differences in Hematocrit Levels during Pregnancy and Preterm Delivery Patricia H. Shiono, Ph.D.
Z01 HD 00371-01	Maternal Dietary Status and Nutritional Status during Pregnancy Lenore J. Launer, Ph.D.
Z01 HD 00372-01	Case-Control Study of the Risk of IVH with High Umbilical Artery Catheters Michael H. Malloy, M.D., M.S.
Z01 HD 00373-01	Calcium Supplementation in Pregnancy to Prevent Preeclampsia and Preterm Birth José G. Rigau, M.D., M.P.H.
Z01 HD 00374-01	Data Coordinating Center for a Study of HIV Infection in Hemophiliacs José G. Rigau, M.D., M.P.H.
Z01 HD 00375-01	Trial of a New Hemophilus Influenzae type b Vaccine José G. Rigau, M.D., M.P.H.
Z01 HD 00376-01	Trial of the Efficacy of a New Pertussis Vaccine-Sweden José G. Rigau, M.D., M.P.H.
Z01 HD 00377-01	Trial of the Safety of a New Pertussis Vaccine-U.S. José G. Rigau, M.D., M.P.H.
Z01 HD 00832-06	Changes in Perinatal and Infant Mortality by Race in Selected U.S. Cities Mary D. Overpeck, M.P.H. and Leslie C. Cooper, Ph.D.

Epidemiology Branch, Prevention Research Program

This year the Epidemiology Branch has continued to worked principally in four areas: studies of risk factors for low birth weight and infant mortality, studies of congenital malformations, nutritional studies of mothers and infants, and, starting this year, studies of vaccine efficacy. A number of these projects have made significant progress this year, and, in addition, branch staff have contributed to selected investigations in other areas of maternal and child health.

Low Birth Weight

Ethnic Differences: The reasons for the large ethnic differences in the incidence of low birth weight and preterm delivery are unknown. A recent study by Lieberman et al. (New England Journal of Medicine 1987; 317:343-8) concluded that differences in hematocrit levels between different ethnic groups may account for these well known but otherwise unexplained differences. However, these authors compared hematocrits of women in preterm labor with those of women in term labor. Normal changes in hematocrit during the third trimester may invalidate this comparison. Branch staff have investigated the hematocrit changes that occur during the third trimester of normal pregnancies and, using methodology that takes these changes into account, have investigated whether anemia explains the racial differences in preterm birth.

This project began with an analysis of the prospectively collected hematocrit data from the Collaborative Perinatal Project. The analysis demonstrated that hematocrit rises, and anemia becomes less common, through the third trimester, and that when women who delivered preterm infants are compared, at comparable times during pregnancy, to women delivering at term, anemia is not a strong predictor of preterm birth. Furthermore, the presence of anemia does little to explain the ethnic differences in preterm birth.

The Branch is obtaining additional data from a subset of the women in the Kaiser-Permanente Birth Defects Study to assess the role of maternal hematocrit/hemoglobin measures in the occurrence of preterm birth. The specific information to be obtained are all measures of hematocrit and hemoglobin levels after the 26th week of gestation among all preterm (N=2,030) deliveries and a group of controls (N=2,030). This information will be used to assess the role of maternal hematocrit in the occurrence of preterm delivery. Preliminary analysis suggests that it will not support the Lieberman hypothesis.

certificates of mothers and children born in Tennessee indicates that the rate of intrauterine growth mediates this effect more strongly than does length of gestation. For example, mothers who weighed 2000-2499 grams at birth are nearly 4 times as likely to have a small for gestational age infant compared to mothers who weighed 4000-4499 grams, but only 1.6 times as likely to give birth to a preterm infant. It was not possible to evaluate the effect of the mother's own gestational age at birth. In order to determine which of these mechanisms is operating, it will be necessary to acquire data sources from the early 1960's in which both length of gestation, birth weight, and other confounding factors were recorded for subjects who can be traced and whose own reproductive performance can be assessed at the present time. Data of this type have been assembled from a health district in Sweden which maintained a low birth weight registry in the 1950's. These data indicate that women who were small for dates at birth are at approximately double risk of giving birth to small for dates infants and nearly threefold increased risk of giving birth to preterm infants. Women who were preterm at birth are not at increased risk for either outcome. Two ongoing projects are studying the intergenerational associations of birth weight, gestational age, and possibly other perinatal complications. One contract with the University of Pennsylvania and Brown University will trace girls who were members of the Philadelphia and Providence cohorts of the Collaborative Perinatal Project (1959-66). The other contract with the University of Southern California and the Psykologisk Institut in Copenhagen will locate girls who were subjects in the Danish Perinatal Study (1959-61). In each study all girls who were born preterm or small for gestational age, and a random sample of controls will be located and their reproductive outcomes determined. Subject tracing and interviewing is currently in progress.

In an additional study of the long-term effects of low birth weight, PRP is collaborating with the Epidemiology Branch of the NIMH to determine whether low birth weight infants and infants who experienced other adverse perinatal events are at increased risk for suicide and injury-related death when they become young adults. In this study, the names of a cohort of young adults whose prenatal and perinatal history has been previously documented in the Collaborative Perinatal Project will be submitted to the National Death Index, and all relevant death certificates will be retrieved. Cause of death will be coded and analyzed.

Smoking: Maternal smoking has been identified as the most important single risk factor for low birth weight that is potentially modifiable. The Smoking Trial of Pregnancy Project (STOP) will be carried out as a randomized clinical trial to evaluate different approaches to smoking cessation within physician practice settings. This project is a collaborative effort between the NICHD and the American College of Obstetricians and Gynecologists. The unit of randomization in STOP will be a practicing physician's population of pregnant women who smoke or

Teratologic and Genetic Problems

The study of periconceptional vitamin use in women having fetuses or infants with neural tube defects has been completed. This study was designed to address the question, "does periconceptional vitamin use reduce the risk of neural tube defects?" A final report on this project has been accepted for publication by the New England Journal of Medicine. The study demonstrates that neither multivitamin use in the periconceptional period nor folate use was associated with any significant reduction in neural tube defects. Comparing 571 women who had a conceptus with a neural tube defect, 546 women who had a conceptus with another malformation or a stillbirth, and 573 who had a normal conceptus, the odds ratio for neural tube defects for those classified as fully supplemented with multivitamins was 0.95 when compared with the abnormal control subjects and 1.00 when compared with the control subjects. Use of folate supplements likewise was no more common in control subjects than in neural tube defects cases. This study indicates that periconceptional use of multivitamin and folate containing supplements is not associated with a decreased risk for neural tube defects in the general population.

The second major question from the Diabetes in Early Pregnancy Study has also been resolved. It has been demonstrated that diabetic women in good metabolic control are not at increased risk for suffering spontaneous abortions. Interestingly, diabetic women who have a poor metabolic control as evidenced by increases in glycosylated hemoglobin or blood glucose are at increased risk for experiencing a miscarriage. This study was published in the New England Journal of Medicine in December 1988.

A new analysis from the Diabetes in Early Pregnancy Study has been completed recently and is now being prepared for publication. The analysis examined the relationship between maternal blood glucose control during pregnancy and the risk of having a macrosomic infant. The relationship between poor glucose control and macrosomia is controversial. We found that poor control, as measured by either glucose or glycosylated hemoglobin, was associated with an increased rate of macrosomia and, most importantly, that non-fasting glucose was the strongest predictor of macrosomia. Moreover, glucose control in the first trimester was shown to predict which women were at high risk for producing a macrosomic infant. Control in the third trimester was most important as a direct determinant of macrosomia risk.

The study of congenital malformations and development in children conceived by in vitro fertilization will appear shortly in the Journal of Pediatrics. This study demonstrated that children born by in vitro fertilization obtain high scores on Bayley Developmental testing; however, these scores are a reflection of their socioeconomic background and in our study were comparable to the scores obtained by matched control subjects. No increased risk for congenital malformations was found in the in vitro fertilization group.

The U.S.-Finland Collaborative Study to examine vitamin levels during pregnancy of women who had children with neural tube defects and matched control subjects is progressing. Blood samples have been collected and shipped to New York City where they will be assayed for folate, vitamin B12, and, if possible, vitamin A. Questionnaires have been developed and interviewing the study subjects in Finland will begin shortly.

Nutrition

The Branch has continued to be involved in several projects relating to nutrition during pregnancy and childhood. As noted above the Longitudinal Study of Perinatal and Nutritional Epidemiology, conducted in Guatemala, has examined height, weight, and weight gain in 17,000 pregnant women in a developing country setting and has related them to subsequent pregnancy outcome. On a sub-set (n=120) of the pregnant women engaged in the Guatemalan Perinatal Risk Factor study body composition was measured longitudinally using bioimpedence and anthropometry. For these women we have described the patterns of change in fat and fat-free mass during pregnancy. Studies of lactose digestion were examined in another group of these women and suggested that lactose tolerance improves during pregnancy. Studies of calcium and iron absorption in pregnancy have been conducted in a separate group of 200 lower class pregnant women in Baltimore.

Dietary supplementation with 1.5 g to 2.0 g elemental calcium per day during pregnancy has been found to reduce blood pressure levels and the frequency of preterm birth in two modest randomized trials. It is proposed to test this effect in a larger 2-3 center collaborative, double-masked, randomized clinical trial in which about 4000 women will be enrolled in the first or second trimester and will take 4 calcium tablets (or placebo tablets) daily. Standardized blood pressure measurements will be obtained at entry, at routine prenatal visits and at least once near term. Urinary protein excretion and pregnancy dating will be carefully recorded. Primary outcomes of the trial will be the frequency of pregnancy induced hypertension and of preterm birth. Birth weight will be an important secondary outcome.

In another project we are examining the relationship between food intake and body composition changes during months six through nine of pregnancy on a sample of women (n=575) from Madura, Indonesia who participated in an energy-supplementation trial. For this we are developing appropriate statistical models to describe the longitudinal changes. Additional analyses are being undertaken to examine the determinants of food intake during pregnancy, with an emphasis on changes that occur over the months of pregnancy.

A prospective survey of maternal employment and breast feeding initiation and duration was conducted among 668 Black and 511 White women who delivered their first child in Washington, D.C. Ninety-one percent of White women (N=511) and 80% of Black women (N=668) reported working during pregnancy. Black women who

Vaccine Trials

Hemophilus influenzae type b is the most common cause of bacterial meningitis in the U.S.; about 13,000 infants and children contract this disease annually. The population at greatest risk consists of children under 2 years of age, but currently licensed vaccines are not recommended for use before age 18 months. The object of this proposal is to evaluate the safety, immunogenicity, and efficacy of a new vaccine developed by NICHD scientists. We plan to obtain information on the preventive effect of a three-dose course of immunization with a new *Hemophilus influenzae* type b (Hib) vaccine (Hib capsular polysaccharide conjugated with tetanus toxoid) on the incidence of Hib invasive disease in children age 2 months at enrollment, and followed for 2-4 years. We propose to conduct a double-masked, randomized, placebo-controlled efficacy trial, with careful surveillance for symptoms of disease, and adverse reactions from the vaccine.

The whole-cell pertussis vaccine currently in use in the U.S. provides insufficient efficacy (80%), limited duration of immunity, and worrisome side effects. We propose to conduct an efficacy trial of a new acellular pertussis (Ptx) vaccine in Sweden (Goteborg), where vaccination with whole-cell vaccine was discontinued in 1979, and where whooping cough is now endemic. We are now conducting a phase 2 study with 120 infants age 7-10 weeks at enrollment, who will be immunized at 3 separate times at two month intervals. A blood sample will be collected from each infant at visit 1 and 3, and 4-6 weeks after the last injection. A blood sample from the mother will also be collected at the first visit. Information will be collected from parents about any reactions the child may have to the vaccine, and any other health events in the follow-up period. Sera will be analyzed for antitoxin and IgG PT levels. Active surveillance for pertussis cases in the Goteborg area is now in effect. If the results of this pilot study are considered satisfactory by the appropriate review committees, we propose to carry out an efficacy trial of the Ptx vaccine. We will try to demonstrate, through a double-masked, controlled, randomized trial, that infants receiving a 3-dose course of Ptx vaccine will have a lower incidence of pertussis than infants who do not receive the vaccine. We will, as in the phase 2 study, immunize infants age 7-10 weeks at enrollment, for 3 separate injections at two month intervals. Bloods will be collected from infants and mothers, as before, and will also be analyzed for antitoxin and IgG PT levels. Active surveillance for reactions and disease will, of course, also be instituted.

To test the safety of the NICHD-developed pertussis vaccine in the U.S., it will be necessary to perform a randomized, double-masked trial with at least 15,000 children immunized at 2, 4, 6, and 18 months of age, and followed for 2 years after the last injection. Data will be collected on incidence of pertussis and adverse effects from immunization.

trimester to term. The incidence of pre-eclampsia in the two groups will be compared. A third study, scheduled to begin later this year, will evaluate the role of antibiotics in the treatment of spontaneous preterm labor. Women in preterm labor of unknown etiology will be randomized to receive ampicillin and erythromycin or corresponding placebos. The effect of these drugs in treating subclinical infection, thereby prolonging pregnancy and averting neonatal morbidity, will be determined.

The investigation of long term complications of growth hormone use is nearing completion. All subjects have now been interviewed and data are now being analyzed to determine how many cases of Creutzfeld-Jacob disease have occurred in growth hormone recipients. In addition a postulated increased risk for leukemia and other childhood cancers is being examined in this population.

The ongoing study of reproductive outcomes in survivors of childhood leukemia has now been approved by the Children's Cancer Study Group and is being submitted to the collaborating institutions for approval by their Institutional Review Boards. A wide range of reproductive issues will be addressed in this study including pubertal development, menarche, fertility, spontaneous abortion in survivors, and congenital malformations in the offspring of survivors. It is also possible that social problems, employment problems and other indirect complications of childhood cancer therapy will be addressed.

A national survey was conducted as a supplement to the National Health Interview Survey to document the health status of children in the U.S. in 1988. Subjects included accidents, injuries, poisonings, other childhood morbidity, child care, family relationships, perinatal events, use of health services, school performance and behavior. The survey is a collaborative effort of NICHD, the Health Resources and Services Administration, Child Trends Inc., the National Center for Health Statistics and the U.S. Census Bureau. The Branch took a very active role in developing the instrument, providing analysis plans and reviewing edit specifications. Data should be available for analysis in December, 1989. Current analysis plans include 1) a comparison of injuries in day care centers to injuries in homes; 2) a description of the health and behavioral status of very low birth weight children; and 3) environmental exposure to cigarette smoke.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00323-09 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: District of Columbia Perinatal Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: H.W. Berendes Director PRP, NICHD

COOPERATING UNITS *(if any)*: Epidemiology Branch, PRP, NICHD (L.C.Cooper)

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☑ (a1) Minors

☑ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

The D.C. Perinatal Study is a case-control study designed to elucidate the factors associated with the delivery of a low birth weight infant to resident mothers in the District of Columbia. The study "cases" were low birth weight infants (<2500 grams) born in participating hospitals. "Controls" were selected as the next race matched normal weight infant (= >2500 grams) delivered at the same hospital. The mothers of the cases and controls were interviewed on the postpartum ward, with data verification obtained through abstraction of medical records. Where possible, prenatal information was verified by using the prenatal information which was attached to the hospital medical record. However, if the hospital medical record did not contain adequate prenatal information arrangements were made to abstract this information from private and public physician's offices where care was received. Data collection began February 1, 1984, and continued until January 31, 1985. The data was collected by SRA Technologies, Inc., of Arlington, Virginia.

In September 1985 SRA returned the data instruments to NICHD due to an inability to complete the contract. Raw data was returned as well as data entered on two data tapes and disk through the Division of Computer Research and Technology (DCRT). It was necessary to re-key all of the data originally submitted by SRA Technologies. One hundred percent of the data have now been keyed. Manuscripts are now in the process of being prepared for submission to peer reviewed journals. Three abstracts were presented at the American Public Health Associations 116th Annual meeting in Boston, Massachusetts, November 13-17, 1988. A number of other speaking engagements have followed or been scheduled.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00325-08 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Neural Tube Defects and Folate

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.L. Mills	Research Medical Officer	EB, PRP, NICHD
Other:	G.G. Rhoads	Head, Epidemiology Branch	PRP, NICHD

COOPERATING UNITS *(if any)*: Biometry Branch, PRP, NICHD (H.Hoffman)

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

- ☑ (a) Human subjects
  - ☑ (a1) Minors
  - ☑ (a2) Interviews
- ☐ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Epidemiology Branch (PRP) is conducting a case-control study in Illinois and California to determine whether the use periconceptional vitamin supplements can reduce the risk of neural tube defects. Women having either a fetus or an infant with a neural tube defect have been ascertained through perinatal networks, vital records, and other sources and were matched to two controls on maternal race and geographic locale. One control is a mother with a normal pregnancy, and the other the mother of an infant with a fetus with a major health problem. Cases and controls were interviewed within 3 months of the end of pregnancy to determine whether those having a conceptus with a neural tube defect are less likely to have used vitamins in the periconceptional period. The data analysis is now complete. A first report on the results of this study has now been submitted for publication. At least one additional report involving the descriptive epidemiology of neural tube defects is in progress and should be written by the end of 1989.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00329-07 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Evaluation of an Intervention Trial to Prevent Low Birth Weight in D.C.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.D. Overpeck	Health Statistician	EB, PRP, NICHD
Other:	G.G. Rhoads	Head, Epidemiology Branch	PRP, NICHD

COOPERATING UNITS *(if any)*: Office of the Director, PRP, NICHD (H.W.Berendes); Greater Washington Research Center, Washington, DC (J.Maxwell); Better Babies Project, Washington, DC (D.Coates).

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

- ☑ (a) Human subjects
  - ☑ (a1) Minors
  - ☑ (a2) Interviews
- ☐ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

The Better Babies Project (BBP) pilot study was a three-year research and demonstration effort funded by private foundations to reduce the rate of low birth weight and associated infant mortality and illness in a specific high risk area of the District of Columbia. The BBP Service Delivery team began collecting data July, 1984, for the project's mini pilot. As a result of the mini pilot findings a number of revisions were made in the forms and interventions. These revised forms and interventions were then developed and piloted. A four year trial of the project began September, 1986 and will end in 1990. The Project has attempted to identify all pregnant women in a high risk area, help link them with existing medical, social, and health services, facilitate their use of these services, and provide health education and social services.

NICHD had funded two contracts for the BBP to assist with the evaluation. Data management and analysis is currently being done by Computer Data Systems, Inc. The D.C. Department of Human Services, Research and Statistics Division, through a contract with NICHD, is providing us information on all pregnant women delivering in the District of Columbia during the period of the project. Analyses of preliminary data should be completed by June 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00331-06 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Diabetes In Early Pregnancy Project (DIEP)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.L. Mills Research Medical Officer EB, PRP, NICHD

COOPERATING UNITS *(if any)*: Cornell Univ.Med.Center, NY (L.Jovanovic); Brigham and Womens Hosp. Boston, MA (L.Holmes); Northwestern Univ.Med.Center, Chicago, IL (J.L.Simpson); Univ.of Pittsburgh, Pittsburgh, PA (J.Aarons); Univ. of Washington, Seattle,WA (R.Knopp).

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.6	0.2

CHECK APPROPRIATE BOX(ES)

- ☑ (a) Human subjects
  - ☑ (a1) Minors
  - ☑ (a2) Interviews
- ☐ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major objectives of the Diabetes in Early Pregnancy Project regarding malformations and early pregnancy losses have now been realized. The study is now entering the second major phase. Two major areas of investigation will now be undertaken. The first will be to look at other effects of diabetes in pregnancy and the second will be to examine the control population in studies of risk factors for early fetal loss. Currently studies are in progress involving the risks of macrosomia associated with diabetic control, the effect of diabetes on risk factors for cardiovascular disease (triglycerides, cholesterol, blood pressure) and genetic factors associated with the risk for congenital malformations in diabetes. Discussions will soon begin on assays to be performed using the blood specimens collected and stored from the Diabetes in Early Pregnancy Project. In addition to our second major publication in the New England Journal of Medicine, data from this project have recently been presented at FIGO and the American Diabetes Association.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00334-06 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Low Birth Weight Across Generations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.A. Klebanoff	Research Medical Officer	EB, PRP, NICHD
Other:	G.G. Rhoads	Head, Epidemiology Branch	PRP, NICHD

COOPERATING UNITS *(if any)*
Office of the Director, PRP, NICHD (H.W.Berendes); World Health Organization, Geneva, Switzerland (O.Meirik); University of Pennsylvania (S.Katz), University of Southern California (B.Mednick)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.65	.65	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

The original description of the association of maternal and infant birth weights was followed by the description of the association between large maternal birth weight and delivery of a macrosomic (>4000 gram) infant. Study of other fetal growth parameters, including length and head circumference, demonstrated that infants of low birth weight mothers were both shorter and lighter than infants of larger mothers, but that the infants were normally proportioned.

In related studies, birth certificates of infants born in Tennessee between 1979 to 1984 were matched with those of their mothers, who were born in Tennessee between 1959 to 1966. Maternal and infant birth weights were again shown to be correlated. In addition, women who were themselves of low birth weight were up to 4 times as likely to have a small for gestational age infant as were women weighing 4000-4499 grams, but the low birth weight women were less than twice as likely to have a preterm infant. A group of Swedish women, born from 1955 to 1965, was studied. Women themselves smaller gestational age at birth were at increased risk of giving birth to both small for gestational age and preterm infants. Women who were preterm at birth were not at increased risk of either outcome.

Follow-up of girls who were born in the 1960's as subjects in the Collaborative Perinatal Project and Danish Perinatal Study is currently underway in order to examine their reproductive histories. Small for gestational age, preterm and control girls will be located and interviewed. Hospital records of their confinements will also be retrieved.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00340-06 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Ethnic Differences in Birth Weight and Length of Gestation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Shiono	Epidemiologist	EB, PRP, NICHD
Other:	G.G. Rhoads	Head, Epidemiology Branch	PRP, NICHD

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.3	.3	0

CHECK APPROPRIATE BOX(ES)

- ☑ (a) Human subjects
  - ☐ (a1) Minors
  - ☑ (a2) Interviews
- ☐ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A contract to obtain a quantifiable description of behavior and lifestyle differences among pregnant women of different ethnic groups which are known to differ in their rates of low birth weight has been awarded to Columbia University and to Northwestern University. The overall goal of this project is to define previously undescribed risk factors affecting birth outcome from pregnant women in the following ethnic groups: American Blacks, Chinese, Mexican-Americans, Puerto Ricans, and Whites. The work scope of the contract includes development of an extensive questionnaire by a multidisciplinary team of experts, pretesting of the interview instruments, interviewing pregnant women from the five groups noted above, and preparing an edited data tape of all responses. The study is reaching the end of the third year. Study instruments have been developed and piloted and recruitment of pregnant women into the study has commenced.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00344-06 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Long Term Health Effects of Infant Formulas Deficient in Chloride

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.H. Malloy	Research Medical Officer	EB, PRP, NICHD
Other:	G.G. Rhoads	Head, Epidemiology Branch	PRP, NICHD

COOPERATING UNITS *(if any)*: Office of the Director, PRP (H.Berendes), Biometry Branch, PRP, NICHD (B.I. Graubard), CRMC, NICHD (A.Willoughby)

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

- ☑ (a) Human subjects
  - ☑ (a1) Minors
  - ☑ (a2) Interviews
- ☐ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During 1978 and 1979 two infant formulas deficient in chloride were marketed in the United States. It has been estimated that a minimum of 20,000 infant years of these formulas were purchased and more than 100 children were reported to the Centers for Disease Control with metabolic and other abnormalities, principally hypochloremic metabolic alkalosis. In a study of 21 of these children at 2 years of age a significant inverse correlation between length of exclusive use of defective formula and cognitive outcome as measured by the Bayley Scales of Infant Development ($r=-.55$, $p=.01$) was noted. In a population-based study which ascertained the infant formulas used by first and second graders attending public school those who were exposed to defective formula scored lower on the general cognitive index and the quantitative scale (McCarthy) than did the children who used other soy formulas.

To substantiate these findings a further study of children is in progress in the metropolitan Washington, DC, area schools. 188 children exposed to deficient formula and 479 matched control children exposed to other soy formulas have been tested. In addition, approximately 39 children with a documented history of hypochloremic metabolic alkalosis resulting from defective formula use were brought to the Washington area for testing. The performance of all these children on a battery of psychological tests have been measured and a careful statistical analysis is underway to look for an effect of exposure to the defective formula with and without documented illness.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00346-05 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Time Trends in the Incidence of Biliary Atresia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.A. Klebanoff Research Medical Officer EB, PRP, NICHD

COOPERATING UNITS *(if any)*

Case Western Reserve University (B.Chatterjee)

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☑ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Extrahepatic biliary atresia is a liver disease presenting in early infancy, manifested by progressive obliteration of the extrahepatic bile ducts. It has been estimated to occur in from one per 8000 to one per 15000 live births, and is the single most common indication for performance of liver transplantation in children. None of the incidence figures is based on a well defined geopolitical region; most estimates of the frequency of this condition are derived from referral centers. Some investigators have suggested a time-space clustering of this condition.

This project has gathered birth certificates and other information on all cases occurring among children born over a period of 12 years in Ohio. Ninety-four (94) cases were identified, corresponding to a rate of 0.5 cases/10,000 births. Cases will be compared to the other births in the state for evidence of changes in incidence and clustering. A number of potential risk factors for the condition will be examined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00352-04 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Studies of Human Immunodeficiency Virus - Related Problems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G.G. Rhoads	Head, Epidemiology Branch	PRP, NICHD
Other:	M.H. Malloy	Research Medical Officer	EB, PRP, NICHD

COOPERATING UNITS *(if any)*: Office of the Director, PRP (H.W.Berendes), HRSA (S.S.Kessel), American Academy of Pediatrics (C.Croft, G.Fleming).

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

- ☑ (a) Human subjects
  - ☑ (a1) Minors
  - ☑ (a2) Interviews
- ☐ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

NICHD/PRP is involved in helping the American Academy of Pediatrics (AAP) develop an education program for pediatricians that deals with developmental sexuality and AIDS. The Academy has designed a program that calls for the development of an educational package by a group of experts in human sexuality, adolescent medicine and human development. The educational package is then to be administered to a randomly selected group of pediatricians. Follow-up of the pediatricians who receive the education program and follow-up of a group of pediatricians who did not receive the protocol will be carried out to determine if the program affected the pediatricians' behavior in the office setting. To date the Program Development Group has formulated a program that will be administered to pediatricians over an initial session of 1.5 days and a one day follow-up session one month later.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00360-03 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Prospective Study of 1st Trimester Use of Bendectin and Malformations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Shiono	Epidemiologist	EB, PRP, NICHD
Other:	M.A. Klebanoff	Research Medical Officer	EB, PRP, NICHD

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.05	.05	0

CHECK APPROPRIATE BOX(ES)

- ☑ (a) Human subjects
  - ☐ (a1) Minors
  - ☑ (a2) Interviews
- ☐ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Most previous studies on this topic used a retrospective case-control design or indirect measures of exposure (pharmacy records). In this prospective study, 31,602 women were asked at their first prenatal visit about the use of Bendectin; 2,711 women reported use in the first trimester. The odds ratio (and 95% interval estimates) for major malformations was 1.05 (0.78-1.40). When individual malformations were evaluated, Bendectin use was statistically associated with microcephaly (5.33 (1.61-17.7)), cataract (5.33 (0.98-29.1)), and lung malformations (4.58 (1.76-11.9)). Since it is not clear whether these associations are due to the use of Bendectin or to the indication (vomiting) for which the drug was prescribed, the association between vomiting and these malformations was studied using previously published data from the Collaborative Perinatal Project. In that study, vomiting was associated with microcephaly (3.3 (1.1, 9.8)) and cataract (3.5 (0.8-16.1)). Vomiting was associated with these two malformations only among nonusers of Bendectin. Lung malformations were not associated with vomiting during pregnancy (1.3 (0.8-2.1)). These data strongly suggest that Bendectin is not associated with these malformations, however the possibility that vomiting is associated with microcephaly and cataract is supported.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00361-03 EB

PERIOD COVERED
October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Child Health Supplement to the 1988 National Health Interview Survey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.D. Overpeck	Health Statistician	EB, PRP, NICHD
Other:	G.G. Rhoads	Head, Epidemiology Branch	PRP, NICHD

COOPERATING UNITS *(if any)*
Biometry Branch, PRP NICHD (H.J.Hoffman); HLB, CRMC, NICHD (P.C.Scheidt); DBSB, CPR, NICHD (V.S.Cain, W.Baldwin); National Center for Health Statistics; Bureau of the Census; Maternal and Child Health, HRSA, Child Trends, Inc. (N.Zill)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS.	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

- ☑ (a) Human subjects
  - ☐ (a1) Minors
  - ☑ (a2) Interviews
- ☐ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This survey provides data on a nationwide representative sample of 20,000 children. Subjects include child care, family relationships, accidents, injuries, poisonings, other childhood morbidity, perinatal events, use of health services, school performance and behavior. It establishes current normative ranges for the U.S. It will provide data for analysis of trends in the U.S. using the 1981 Child Health Supplement for comparisons. The survey was conducted by the U.S. Census Bureau for the National Center for Health Statistics during the 1988 calendar year. Preliminary data will be available by September, 1989. Final data should be ready for analysis by December, 1989.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00362-03 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Nutritional Aspects of Perinatal Epidemiology in Central America

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: José Villar Expert EB, PRP, NICHD

COOPERATING UNITS *(if any)*: Office of the Director, PRP, NICHD (H.J. Hoffman)

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	0.8	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study attempts primarily to develop a simple instrument, empirically produced for the identification of mothers at rigk of delivering a LBW infant. Longitudinal data are available for selecting variables at different points during pregnancy. Sample size of the total population is 17,000. The risk score is developed in a random sample of 8000 patients and tested in the remaining group. Furthermore, the following projects are performed using this source of data:

- Epidemiology of subgroups of IUGR infants and their neonatal morbidity (paper submitted).

- Physical activity and work during pregnancy and pregnancy outcome (paper submitted).

- Protozoan and helminthic infections during pregnancy and its effect on birth weight (paper submitted).

- Lactose malabsorption during pregnancy: A longitudinal study (paper published).

- Body composition and physical activity during pregnancy and birth weight (paper submitted).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00363-02 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: NICHD Smoking Trial of Pregnant Women (STOP)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.C. Cooper	Nurse Epidemiologist	EB, PRP, NICHD
Others:	P.H. Shiono	Epidemiologist	EB, PRP, NICHD
	G.G. Rhoads	Head, Epidemiology Branch	PRP, NICHD

COOPERATING UNITS *(if any)*: Office of the Director, PRP, NICHD (H.W.Berendes)

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.20	.20	0

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☑ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The STOP Project will be carried out as a randomized clinical trial to evaluate different approaches to smoking cessation within physician practice settings. This project is a collaborative effort between the NICHD and the American College of Obstetricians and Gynecologists. The unit of randomization in STOP will be a practicing physician's population of pregnant women who smoke or have recently stopped smoking. Physicians will be solicited to volunteer to take part in this randomized study.

There will be two major phases to the STOP Project - a pilot and a formal trial. The objective of the pilot study is to develop the protocol for the STOP study, develop all study materials (pamphlets, study forms, manual of operations, etc.), recruit private physicians who will assist us in finalizing the study protocol and materials, assist in the development of all quality control procedures, develop all necessary data management materials (data entry programs, SAS data sets, edit specifications for all data, analysis of the pilot) and train the contractor selected to run the formal trial in all aspects of the study. All study materials (forms, urine testing, pamphlets etc.) will be modified to be easily incorporated into the daily routines of private physicians' offices.

The desired result of the pilot will be to have all necessary forms, materials, etc. complete and ready for use in the formal STOP study.

At this time a RFC is being prepared for the formal trial.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00365-02 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: A Randomized Clinical Trial of Umbilical Artery Catheter Placement

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.H. Malloy	Research Medical Officer	EB, PRP, NICHD
Other:	G.G. Rhoads	Head, Epidemiology Branch	PRP, NICHD

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

- ☑ (a) Human subjects
  - ☑ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was formulated to determine if very low birth weight infants who receive umbilical artery catheters that are placed high in the thoracic aorta (T6-T8) are at higher risk of intraventricular hemorrhage than are infants that receive an umbilical artery catheter placed low in the abdominal aorta (L4-L5). We propose to randomize infants to receive either a high or low catheter and then to review the incidence of intraventricular hemorrhage. The project will enroll a total of 700 infants in 12 neonatal intensive care units beginning in 1989. Ten of 12 nurseries solicited to participate in the project have agreed to participate as of this writing. A contract for a Data Coordinating Center has been awarded to Scientific Applications Co. Randomization of infants should begin in the Fall of 1989.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00366-02 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Survey of Pregnancy Outcomes Among Medical Residents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Shiono	Epidemiologist	EB, PRP, NICHD
Others:	M.A. Klebanoff	Senior Staff Fellow	EB, PRP, NICHD
	G.G. Rhoads	Head, Epidemiology Branch	PRP, NICHD

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.10	.10	0

CHECK APPROPRIATE BOX(ES)

- ☑ (a) Human subjects
  - ☐ (a1) Minors
  - ☑ (a2) Interviews
- ☐ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effects of stressful employment on pregnancy outcome will be examined in this study of pregnancy outcomes among medical residents. Several studies of paid employment by women during pregnancy have shown an increased risk of both preterm birth and low birth weight associated with strenuous occupations. However, the majority of studies show no increased risk. None of the previous studies were able to control adequately for the socioeconomic status of the women, and in many instances improper controls were used. Women who become pregnant during medical residency are in many respects an optimal group in which to study this issue. They are universally highly educated and in many respects of high socioeconomic status, yet their occupation is highly stressful and physically demanding. For this reason, the effects of a mentally and physically demanding occupation can be studied independently of socioeconomic status. Spouses of male residents comprise an appropriate control group, as they are also of high socioeconomic status, but in most cases have less strenuous occupations than that of a medical resident.

We hypothesize that the mentally and physically strenuous occupation of residency adversely effects the pregnancy outcomes of female residents, as compared to the pregnancy outcomes of spouses of male residents. The proposed study will examine pregnancy outcomes among a cohort of recent medical school graduates. Approximately 10,000 residents in their third post-graduate year (all of the women residents and a 50% random sample of male residents) will be surveyed to determine the pregnancy outcomes of the female residents and spouses of male residents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00368-02 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Vaginal Delivery of Very Low Birth Weight Infants: Association with Day 1 Deaths

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.H. Malloy	Research Medical Officer	EB, PRP, NICHD
Other:	G.G. Rhoads	Head, Epidemiology Branch	PRP, NICHD

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☑ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project involved the analysis of linked birth and death certificates from Missouri for the years 1980-1984. The 200,000 births occurring during this period are being studied to determine whether or not cesarean sections performed for very low birth weight infants have any advantages over vaginal delivery after adjusting for various complications of delivery. Our preliminary analysis suggests that the vaginal delivery of a very low birth weight infant carries a greater risk of day 1 death than does cesarean section. However, the advantage is lost during the first week of life when those infants delivered by cesarean section have a higher death role than those delivered vaginally. We suspect that the lower first day death rate for infants delivered by cesarean section is related to the greater attention and resuscitative efforts these infants receive. The study has been accepted for publication by the Journal of the American Medical Association.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00369-01 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Adverse Perinatal Events and Subsequent Injury-related Death

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.A. Klebanoff Research Medical Officer EB, PRP, NICHD

COOPERATING UNITS *(if any)*

ISI, Inc. Alexandria, VA; Epidemiology Branch, NIMH (M. Farmer)

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.15	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several published studies have indicated that children experiencing asphyxia during the perinatal period are at increased risk of subsequent adolescent suicide. However, these studies used retrospectively ascertained data and were unable to control for the adverse social conditions that often accompany perinatal difficulties.

In the first phase of this project, the names of the approximately 55,000 children who were born during 1959-66 as subjects in the Collaborative Perinatal Project will be computerized. During the second phase, the computerized names and other appropriate identifying information will be submitted to the National Death Index, and all subjects who have died will be identified. In the third phase, the death certificates of these subjects will be obtained and cause of death recorded. Since the Collaborative Project collected extensive data about the subjects' prenatal, perinatal, and childhood histories, it will be possible to study prospectively the relationship between adverse perinatal events and subsequent risk of death, as well as cause of death.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00370-01 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Ethnic Differences in Hematocrit Levels during Pregnancy and Preterm Delivery

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Shiono	Epidemiologist	EB, PRP, NICHD
Other:	M.A. Klebanoff	Research Medical Officer	EB, PRP, NICHD

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.20	.20	0

CHECK APPROPRIATE BOX(ES)

- ☑ (a) Human subjects
  - ☐ (a1) Minors
  - ☑ (a2) Interviews
- ☐ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study is to assess the role of maternal hematocrit/hemoglobin measures in the occurrence of preterm birth. A recent study by Lieberman et al. (New England Journal of Medicine 1987; 317:343-8) concluded that differences in hematocrit levels between different ethnic groups may account for the well known but otherwise unexplained differences in the risk of preterm birth between the ethnic groups. One phase of this project analyzed prospectively collected hematocrit data from the Collaborative Perinatal Project. The second phase of this project will obtain additional data from a subset of women in the Kaiser-Permanente Birth Defects Study to assess the role of maternal hematocrit/hemoglobin measures in the occurrence of preterm birth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00371-01 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Maternal Dietary Status and Nutritional Status during Pregnancy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L.J. Launer Epidemiology Staff Fellow EB, PRP, NICHD

COOPERATING UNITS *(if any)*

Department of Biostatistics, Johns Hopkins University (K-Y. Liang, L. Madger)

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.75	0.75	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study attempts to develop appropriate statistical models to examine the longitudinal relationship between food intake and body composition changes during months 6 through 9 of pregnancy.

- The determinants of dietary intake during pregnancy among a group of rural women from Indonesia (data analysis in progress).

- The application of longitudinal statistical models to examine biological changes during pregnancy (data analysis in progress).

- The relationship between dietary status and body composition changes during pregnancy among a group of rural women from rural Indonesia (data analysis in progress).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00372-01 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Case-Control Study of the Risk of IVH with High Umbilical Artery Catheters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.H. Malloy Research Medical Officer EB, PRP, NICHD

COOPERATING UNITS *(if any)*

University of Texas Medical Branch (J.Richardson)

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was carried out to justify the need for a randomized clinical trial of umbilical artery catheter placement. Dr. Joan Richardson at the University of Texas Medical Branch reviewed two years worth of cases of intraventricular hemorrhage (IVH) in infants weighing less than 1500 grams and controls matched to the cases by birth weight and date of birth. The prevalence of high catheters in infants with intraventricular hemorrhage was 3 times higher than in controls suggesting an association between high catheters and IVH.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00373-01 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Calcium Supplementation in Pregnancy to Prevent Preeclampsia and Preterm Birth

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.G. Rigau Senior Epidemiologist EB, PRP, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.21	0.21	0

CHECK APPROPRIATE BOX(ES)

- ☑ (a) Human subjects
  - ☑ (a1) Minors
  - ☑ (a2) Interviews
- ☑ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dietary supplementation with 1.5 g to 2.0 g elemental calcium per day during pregnancy has been found to reduce blood pressure levels and the frequency of preterm birth in two modest randomized trials. It is proposed to test this effect in a larger 2-3 center collaborative, double-masked, randomized clinical trial in which about 4000 women will be enrolled in the first or second trimester and will take 4 calcium tablets (or placebo tablets) daily. Standardized blood pressure measurements will be obtained at entry, at routine prenatal visits and at least once near term. Urinary protein excretion and pregnancy dating will be carefully recorded. Primary outcomes of the trial will be the frequency of pregnancy induced hypertension and of preterm birth. Birth weight will be an important secondary outcome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00374-01 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Data Coordinating Center for a Study of HIV Infection in Hemophiliacs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.G. Rigau Senior Epidemiologist EB, PRP, NICHD

COOPERATING UNITS *(if any)*

PAMA, CRMC (A. Willoughby)

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.125	0.125	0

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☑ (a1) Minors

☑ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Multicenter Study of the Natural History of HIV Infection in Hemophilic Children (also titled, for brevity's sake, the Hemophilia Growth and Development Study - HGDS) consists of a network of 11 clinical centers for patient recruitment and a 4-year follow-up, and a data coordinating center for computer and logistical support, forms development, and data entry and limited periodic analyses. The object of the study is to monitor and compare the health of three cohorts of children: HIV-infected hemophiliacs, non-infected hemophiliacs, and non-hemophiliac, non-infected male siblings of hemophiliacs.

For each study participant a conprehensive data base of medical history and health events will be put together, with emphasis on the prospective collection of data on body growth, and endocrine and neuropsychological development. At the conclusion of the first year of operation (May 1988 - April 1989), the data coordination center for HGDS (New England Research Institute, Inc.) has participated in the finalization of the study design, the standardization of protocols and forms for data flow, recruitment, baseline follow-up and laboratory examinations, and the training of clinic personnel who will participate in the study. In the first 3 months of recruitment (March-May 1989) 49 patients have been enrolled and evaluated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00375-01 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Trial of a New Hemophilus Influenzae type b Vaccine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	J.G. Rigau	Senior Epidemiologist	EB, PRP, NICHD
Other:	D.A. Bryla	Statistician	BB, PRP, NICHD

COOPERATING UNITS *(if any)*

IRP/LDMI (R. Schneerson)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.125	0.125	0

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☑ (a1) Minors
- ☑ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hemophilus influenzae type b is the most common cause of bacterial meningitis in the U.S.; about 13,000 infants and children contract this disease annually. The population at greatest risk consists of children under 2 years of age, but currently licensed vaccines are not recommended for use before age 18 months. The object of this proposal is to evaluate the safety, immunogenicity, and efficacy of a new vaccine developed by NICHD scientists. We plan to obtain information on the preventive effect of a three-dose course of immunization with a new Hemophilus influenzae type b (Hib) vaccine (Hib capsular polysaccharide conjugated with tetanus toxoid) on the incidence of Hib invasive disease in children age 2 months at enrollment, and followed for 2-4 years. We propose to conduct a double-masked, randomized, placebo-controlled efficacy trial, with careful surveillance for symptoms of disease, and adverse reactions from the vaccine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00376-01 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Trial of the Efficacy of a New Pertussis Vaccine - Sweden

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.G. Rigau	Senior Epidemiologist	EB, PRP, NICHD
Other:	D.A. Bryla	Statistician	BB, PRP, NICHD

COOPERATING UNITS *(if any)*: IRP/LDMI (J. Robbins)

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.17	0.17	0

CHECK APPROPRIATE BOX(ES)

- ☑ (a) Human subjects
  - ☑ (a1) Minors
  - ☑ (a2) Interviews
- ☐ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The whole-cell pertussis vaccine currently in use in the U.S. provides insufficient efficacy (80%), limited duration of immunity, and worrisome side effects. We propose to conduct an efficacy trial of a new acellular pertussis (Ptx) vaccine in Sweden (Goteborg), where vaccination with whole-cell vaccine was discontinued in 1979, and where whooping cough is now endemic.

We are now conducting a phase 2 study with 120 infants age 7-10 weeks at enrollment, who will be immunized at 3 separate times at two month intervals. A blood sample will be collected from each infant at visit 1 and 3, and 4-6 weeks after the last injection. A blood sample from the mother will also be collected at the first visit. Information will be collected from parents about any reactions the child may have to the vaccine, and any other health events in the follow-up period. Sera will be analyzed for antitoxin and IgG PT levels. Active surveillance for pertussis cases in the Goteborg area is now in effect.

If the results of this pilot study are considered satisfactory by the appropriate review committees, we propose to carry out an efficacy trial of the Ptx vaccine. We will try to demonstrate, through a double-masked, controlled, randomized trial, that infants receiving a 3-dose course of Ptx vaccine will have a lower incidence of pertussis than infants who do not receive the vaccine.

We will, as in the phase 2 study, immunize infants age 7-10 weeks at enrollment, for 3 separate injections at two month intervals. Bloods will be collected from infants and mothers, as before, and will also be analyzed for antitoxin and IgG PT levels. Active surveillance for reactions and disease will, of course, also be instituted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00377-01 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Trial of the Safety of a New Pertussis Vaccine - U.S.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.G. Rigau	Senior Epidemiologist	EB, PRP, NICHD
Other:	D.A. Bryla	Statistician	BB, PRP, NICHD

COOPERATING UNITS *(if any)*: Lab of Developmental & Molecular Immunity, NICHD (J. Robbins)

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0.08	PROFESSIONAL: 0.08	OTHER: 0

CHECK APPROPRIATE BOX(ES)

- ☑ (a) Human subjects
  - ☑ (a1) Minors
  - ☑ (a2) Interviews
- ☐ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To test the safety of the NICHD-developed pertussis vaccine, it will be necessary to perform a randomized, double-masked trial with at least 15,000 children immunized at 2, 4, 6, and 18 months of age, and followed for 2 years after the last injection. Data will be collected on incidence of pertussis and adverse effects from immunization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00832-06 EB

PERIOD COVERED: October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Changes in Perinatal and Infant Mortality by Race in Selected U.S. Cities

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.D. Overpeck	Health Statistician	EB, PRP, NICHD
	L.C. Cooper	Nurse Epidemiologist	EB, PRP, NICHD
Other:	G.G. Rhoads	Head, Epidemiology Branch	PRP, NICHD

COOPERATING UNITS *(if any)*: Biometry Branch, PRP, NICHD (H.J.Hoffman)

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.30	.25	0.05

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☑ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study describes differences in perinatal mortality and age at death in 59 large cities from 1972 through 1981, during this period of rapid change in technology and medical management of high risk pregnancies.

It explores whether high rates of neonatal mortality in certain cities can be explained by shifts in mortality from the late fetal to the neonatal period and compares differences in perinatal experience according to age at death by race and city size. A secondary analysis of data sets provided by the National Center for Health Statistics was done based on 100 percent reporting of perinatal deaths. Review of fetal death rates from 24 weeks gestational age and of neonatal deaths for the periods, 1-7, and 8-27 days is being used to examine potential reporting differences among cities and shifting of neonatal deaths into the latter period. These data have not been available publicly for analysis. The analysis provides an improved standard for comparison of perinatal mortality in differing geographic sites.

PREVENTION RESEARCH PROGRAM (PRP)

ZO1 HD 00343-06 — Effect of Westernization on Infant Feeding Patterns Among the Negev Bedouins
Heinz W. Berendes, M.D., M.H.S.

ZO1 HD 01700-02 — Study of the Efficacy of IVIG in HIV Infected Children
Heinz W. Berendes, M.D., M.H.S.

ZO1 HD 01701-01 — Evaluation of the Impact of a Model Prenatal and Followup Program in Baltimore
Heinz W. Berendes, M.D., M.H.S.
and Allen Herman

Office of the Director, Prevention Research Program

The Prevention Research Program conducts investigations in the field of perinatal epidemiology which encompasses many key issues in maternal and child health, such as determinants of perinatal and infant mortality and of sudden infant death syndrome, factors associated with intrauterine growth retardation or preterm delivery, investigations of specific risk factors associated with congenital malformations and also nutritional aspects of pregnancy and of infant feeding practices and their effects on growth and development during infancy. The program is also involved at the present time in several clinical trials and community based interventions. These research projects are conducted by the Office of the Director and by the Epidemiology and Biometry Branches of the Prevention Research Program.

In keeping with the congressional mandate for greater emphasis on research leading to prevention of disease, this program is developing increased capability in the area of prevention research, and specifically health education/health promotion interventions in the area of maternal and child health, by the recruitment of a senior individual with extensive experience in this field. We expect that this activity will come to fruition during FY 90.

In collaboration with the Pediatric, Adolescent and Maternal AIDS Branch, the clinical trial of the efficacy of intravenous gamma globulin in the treatment of symptomatic children infected with the human immunodeficiency virus has continued. It tests the hypothesis that intravenous immunoglobulin when administered every 28 days will significantly reduce the proportion of the treatment group who develop at least one invasive or serious bacterial infection or die during the two year treatment period when compared to the control group of HIV infected children who are receiving an intravenous albumin placebo every 28 days. The trial is now conducted in 30 hospitals around the country and has enrolled more than 300 children as of July 31, 1989. This study has progressed very satisfactorily and has excellent compliance of children, their parents or caretakers in adhering to the follow up schedule. This trial shows distinctly and convincingly that community based hospitals in a variety of settings and in different parts of the country, including Puerto Rico, as well as academic institutions, can implement complex clinical protocols of clinical trials of children with HIV infection. This demonstration is of great importance in view of the fact that many HIV infected children are under the care of community based hospitals and not major academic institutions.

An infant feeding study among Bedouin Arabs who live in the Negev in Israel and are receiving their care at the Soroka Medical

Center, which is part of Ben Gurion University in Beer Sheva, has been completed. This population based, prospective study has identified a number of risk factors which reduce the ability of mothers to exclusively breast feed shortly after birth or curtail the duration of exclusive breastfeeding. A number of these had to do with the hospital practice where these women delivered. It became apparent that mothers delivered by cesarean section were discouraged from breastfeeding, although other studies have shown that women recover from the immediate effects of a cesarean section and usually within 24 to 36 hours after the operation are perfectly capable of initiating breastfeeding. Other variables included birth weight of more than eight pounds and low birth weight, although healthy, that is without major illness or malformations. There was a distinct reduction in exclusive breastfeeding among young nulliparae women. There also was a change in the rate of exclusive breastfeeding depending on season of year with children born during the summer months experiencing a higher rate of exclusive breastfeeding than children born during the winter months. It turned out that this was related to seasonal variation in births with a significant increase in deliveries occurring during the winter months as compared to the summer months. This apparently overloaded the newborn nurseries in the winter months and constituted a heavy burden on the personnel staffing these facilities resulting in less access of mothers to their children.

A follow up of children beyond six months of age revealed a high rate of stunting not different than that seen in other developing countries. One of the major risk factors of stunting past six months is stunting prior to six months suggesting that the prevention of stunting requires interventions which have to start during the first few months of life. Not unexpectedly we found high correlations between respiratory and gastrointestinal morbidity and type of infant feeding practice. Higher rates of respiratory and gastrointestinal morbidity are associated with other than exclusive breastfeeding, and in turn respiratory and gastrointestinal morbidity was related to the risk of stunting.

We conducted a workshop in May of this year with our co-investigators at Ben Gurion University in Beer Sheva to present the key findings from this project and discuss the implications with faculty members of Ben Gurion University, specifically in obstetrics and pediatrics, policy makers in Israel and Arab Bedouin physicians and other interested scientists and health professionals. In a subsequent group discussion, we specifically focused on the identification of interventions which would address the key risk factors identified in this study which are amenable to intervention.

We are collaborating with the Baltimore City Health Department in the development of an intensive outreach and case management program which is aimed at helping high risk pregnant women give birth to healthy infants. This is a multi-risk factor intervention which will, among other things, provide social

support, outreach to enhance participation in prenatal care and compliance and specific interventions which address smoking, drug and alcohol abuse as well as poor nutrition. The project will be geographically based and will be located in an area defined by census tracts in West Baltimore. The area chosen is characterized by a high rate of drug abuse among pregnant women which is estimated to be approximately 30% with a good deal of this, as in other inner city population, due to the use of "crack" cocaine. In view of the increasing evidence that "crack" cocaine is a risk factor for preterm delivery, an intervention to reduce "crack" cocaine use during pregnancy is a major component of this study. Support for this study is derived from private foundations as well as the city of Baltimore and donated staff time of a number of individuals from the City Health Department, the State Health Department and the University of Maryland. The Prevention Research Program is providing technical expertise to the design and development of this project and has assumed responsibility for the evaluation of the intervention. This project will begin later on this year or early next year.

We are continuing our collaboration with the Department of Community Health at the Aga Khan University in Karachi, Pakistan in the conduct of a broad based study of maternal and infant health. One component of the study consisting of a Maternal Infant Mortality Survey is currently proceeding in select areas of Karachi and will provide for the first time population based data on maternal and on infant mortality in Pakistan. This is a pilot for a major maternal and infant mortality survey in Pakistan to be done subsequently in four different sites in the four provinces which make up Pakistan. The total survey will include 60,000 households chosen in such a manner that they present both urban and rural areas in different parts of the country. Another component of the study deals with a cross-sectional survey of pregnant women in various Katchi Abadies in Karachi to identify major risk factors associated with poor birth outcome as baseline information for interventions and also follow up of children to two years of age. The follow up of children will emphasize growth monitoring to identify growth failure and will test various interventions to improve nutrition in young children.

In collaboration with the Indian Council of Medical Research, we conducted a workshop on perinatal determinants of child survival in New Delhi in February 1989 and are preparing the proceedings at the present time for publication later on this year. This workshop was attended by 40 scientists from the Indian side, representing a wide variety of disciplines which included obstetrics and pediatrics, community medicine, infectious diseases, nutrition, sociology, economics, statistics, and 10 scientists from the U.S. side. Presentations covered determinants of infant mortality with particular emphasis on nutritional factors, infections during pregnancy and the perinatal period and social, demographic and cultural factors. A discussion of the determinants of neonatal and postnatal mortality underlined the problems of lack of basic neonatal care, of tetanus neonatorum

and its prevention, diarrheal diseases and the use of ORT, measles and measles vaccination and the early diagnosis and treatment of acute respiratory infections in the young child. We highlighted in one presentation the use of the risk approach of basic and maternal and child health care as an effective tool for the evaluation of the current health status of a community which may be helpful in the identification of key elements which require correction and resolution.

The interaction during the workshop after the formal presentations resulted in the identification of numerous interventions of interest to the Indian Council of Medical Research.

As a result of this workshop, there will be periodic consultations between the two sides at intervals of 12 to 18 months which will be held either in India or the United States to exchange ideas and protocols in maternal and child health.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00343-06 PRP

PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effect of Westernization on Infant Feeding Patterns Among the Negev Bedouins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: H.W. Berendes Director PRP, NICHD

COOPERATING UNITS *(if any)*

Cancer Prevention Branch, NCI, NIH (M.R. Forman); Demographic and Behavioral Sciences Branch, CPR, NICHD (B. Graubard); Ben Gurion University of the Negev, Beer Sheva, Israel (L. Naggan)

LAB/BRANCH

Office of the Director, PRP

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.30	.15	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This is a study of infant feeding practices among Bedouin tribes residing in the Negev, Israel. The objectives are: the evaluation of changes in infant feeding practices during the first year of life and their relationship to physical growth of children and on gastrointestinal and respiratory diseases during the first year of life.

The information obtained covers 5,000 mother-infant pairs. Two samples have been identified; one was identified at birth and a subsample of these births was followed for a period of 5-8 months. Another sample of children was identified at 6 months of age and followed prospectively to 18 months of age.

The analysis of the data is now complete and manuscripts have been prepared or are being prepared and submitted for publication on the following topics: Perinatal Determinants of Infant Feeding Practices, Seasonality of Births Among Bedouin Arabs Residing in the Negev Desert of Israel, Physical Growth as Related to Choice of Infant Feeding Practice at Birth and Changes During the First Year of Life, Morbidity, Especially Respiratory and Gastrointestinal Morbidity, as Related to Growth and Development as well as a major methodological paper.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01700-02 PRP

PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of the Efficacy of IVIG in HIV Infected Children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H.W. Berendes	Director	PRP, NICHD
Others:	A. Willoughby	Head	PAMA, CRMC, NICHD
	J. Rigau	Senior Epidemiologist	EB, PRP, NICHD

COOPERATING UNITS *(if any)*

PAMA, CRMC, NICHD (R. Nugent); BB, PRP, NICHD (G. Reed); OD, CRMC, NICHD (S. Yaffe)

LAB/BRANCH

Office of the Director, PRP

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The IVIG trial network currently consists of over 25 collaborating hospitals, which have enrolled over 243 children in the first year of recruitment (March 1, 1988 - February 28, 1989). The protocol specifies a 2-year follow-up of 366 children. The study has been accepted very well by parents, children and their guardians, as evidenced by a compliance rate of over 95%.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01701-01 PRP

PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Evaluation of the Impact of a Model Prenatal and Followup Program in Baltimore

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	H. W. Berendes	Director	PRP, NICHD
Other:	A. Herman	Visiting Scientist	PRP, NICHD

COOPERATING UNITS *(if any)*

Department of Health, Baltimore City (Tom Coyle)

LAB/BRANCH

Office of the Director, PRP

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

This project is an intensive outreach and case management program which is aimed at helping high risk pregnant women give birth to healthy infants. It is a multi-risk factors intervention which will provide social support, outreach to enhance participation in prenatal care and compliance and special interventions to address smoking, drug and alcohol use as well as poor nutrition. The project will be geographically based and will be located in an area defined by census tract in West Baltimore.

The initiative for this study comes from the Baltimore City Health Department and the Mayor of the City who has invited us to assist in the design and evaluation of this project.

Outcome measures to determine the impact of this project include rate of low birth weight and mean birth weight, the rate of adverse health habits and change in these habits during pregnancy, change in knowledge and attitudes toward more appropriate health behaviors during pregnancy and improved parenting skills.

The project is in the design phase and is scheduled to start about November 1, 1989. Funding for the study is provided from various private foundations to the Health Department of the City of Baltimore, as well as funding from the City itself.